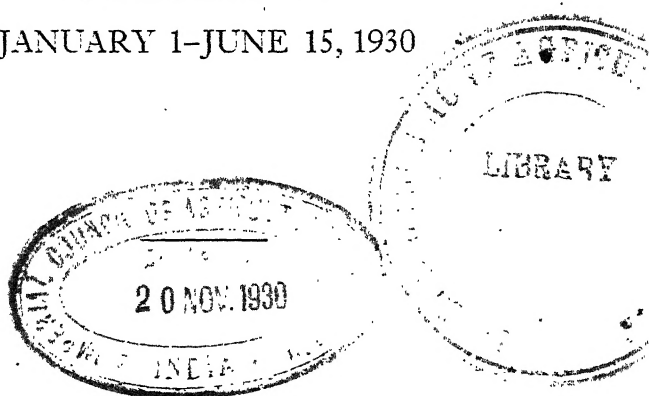


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ERRATA AND AUTHOR'S EMENDATIONS

- Page 25, line 12, "of" should be "or."
- Page 32, eighth line from bottom, "Hybrid 133" should be "Hybrid 143."
- Page 40, Table 2, fourth column, under "Dry matter," first line from bottom, "58.0" should be "58.8."
- Page 40, Table 2, fifth column, under "Organic matter," first line from bottom, "58.8" should be "58.7."
- Page 41, Table 3, fourth column, under "CO₂," line 16, "4,847.9" should be "4,847.8."
- Page 50, Table 10, delete "eliminated" immediately following "0.707" in heading of table.
- Page 55, Figure 1, in legend, "affteed" should be "affected."
- Page 84, Table 2, last column, delete "per day" in box head.
- Page 174, line 9, "improved" should be "reduced."
- Page 174, under "Tests in 1928," lines 8 and 9, "increased it 2 per cent for" should be "had no effect on."
- Page 188, second line from bottom, "141" should be "161."
- Page 189, line 11, "141" should be "161."
- Page 258, line 6, "CO 1" should be "CO₂."
- Page 398, Table 3, second column, under "Midseason," "U. S. D. A. No. 672" should be "U. S. D. A. No. 682."
- Page 451, footnote 9, line 1, "5 gm." should be "0.5 gm."
- Page 579, Table 2, third column, under "s," footnote reference figure "4" should be "3."
- Page 579, footnote 4 (b), " $y = 3.881 + 0.324 x$ " should be " $y = -3.881 + 0.324 x$."
- Page 700, footnote a to Table 4, "or" should be "of."
- Page 768, line 4, "chlorate" should be "chloride."
- Page 783, in legend for Figure 4, "X1,500" should be "X350."
- Page 917, line 5, under "Botanical Description of *Bikukulla formosa*," "orolla" should be "Corolla."
- Page 956, tenth line from bottom, "Berkfeld" should be "Berkefeld."
- Page 1031, line 24, "Noxtoxus" should be "Notoxus."
- Page 1047, paragraphs 1 and 2, immediately following Table 5, should be inserted immediately after Table 3 page 1044.
- Page 1051, line 15, "Valles" should be "Vallese."

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No. 1

BACTERIAL BLIGHT OF POPPY CAUSED BY BACTERIUM PAPAVERICOLA, SP. NOV.¹

By MARY K. BRYAN, *Associate Pathologist, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*,
and FRANK P. MCWHORTER, *Pathologist, Virginia Truck Experiment Station*

INTRODUCTION

A striking disease of the Shirley poppy (*Papaver rhoeas* L.) was observed by the junior writer while visiting the Council plant farm, Franklin, Va., in May, 1927. Since the disease was decidedly destructive, probably of bacterial etiology, and apparently unlike any heretofore-described poppy disease, a rather full description of it was immediately sent to the Office of Mycology and Disease Survey of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C. The present paper is a preliminary report intended to establish the bacterial nature of the disease and to describe the pathogene as a new species of Bacterium.

HISTORY OF THE DISEASE IN THE TYPE LOCALITY

Through visits to the garden of the Council plant farm, Franklin, Va., and by correspondence with its owner, the writers are able to present the following brief history of the garden plot from which the type material was collected.

Seeds of the Shirley poppy were planted in the spring of 1926. According to the grower, a few of the plants developed the disease during the latter part of the first (1926) season; at that time the disease was destructive to the individual infected plants, but not to the plot as a whole. The 1926 plants reseeded the area and served as a source for the 1927 crop. The 1927 plants, when small, were given a generous application of sheep manure, which, according to the grower, may have predisposed the plants to the disease, for during the 1927 season it was conspicuous even before the plants reached the flower-bud stage. By the middle of the blooming season many of the plants were killed, and those remaining were made unsightly by numerous black spots on leaves and buds. While the plants were in this condition the owner burned over the area, hoping to eradicate the disease and to prevent the poppies from reseeding. Some escaped, however, for in 1928 a few came up; these were left for a while, but since they developed the disease long before blooming time, they too were burned.

Type material was collected from this plot by the writers in May, 1927, and later filed in the herbarium of the Office of Mycology and Disease Survey.

¹ Received for publication Apr. 29, 1929; issued January, 1930. This paper resulted from a joint project carried out by the Virginia Truck Experiment Station, Norfolk, Va., and the Bureau of Plant Industry, U. S. Department of Agriculture.

IMPORTANCE AND GEOGRAPHICAL DISTRIBUTION

The behavior of this disease on poppies at Franklin, Va., points to its having great possible economic importance. It not only made the plants unsightly by conspicuous black spotting of the leaves and stems but also killed many of them. Moreover, later infection tests have shown that it is readily transmissible.

The writers have observed this disease on the Shirley variety at the Franklin locality only. That it has occurred elsewhere in the United States is shown by a report of Clinton,² in 1909 and by herbarium material kindly furnished by him. Clinton's collection of the leaf spot form of the disease was made at Westville, Conn. In appearance, the lesions are identical with those on the Virginia specimens. Bacteria were found in great abundance in the spots, but, as was expected, attempts to isolate them from this herbarium material failed.

In addition to the Shirley poppy, the Oriental poppy (*Papaver orientale* L.) must be considered as susceptible. Mature pods and leaves of Oriental poppies with lesions similar to those on Shirley poppy were collected at North, Va., by the junior writer in June, 1927. The organism isolated from these leaves showed slight cultural variations from the Shirley poppy strain, but when inoculated into Shirley poppies it produced lesions characteristic of the disease.

SYMPTOMATOLOGY

The following discussion is based on the examination of plants naturally infected in the type locality and on observation of plants subsequently inoculated with pure cultures of the organism. Lesions were abundant on all the aerial parts, but for convenience in discussion the symptoms on leaf, stem, and floral parts are presented separately.

EFFECT ON LEAVES

The general appearance of infected leaves is shown in Figures 1 and 2. The intensely black spots suggest those produced on rose leaves by *Diplocarpon rosae* Wolf.

Young infections are first evident as minute water-soaked areas; these soon darken into definite spots bounded by a hyaline or water-soaked ring. This ring is seldom evident in older spots.

The size and the shape of mature spots are affected by the distribution of the primary lesions on the leaf. When scattered they are distinctly circular, clearly defined, often zonate (fig. 3), and may attain a diameter of 3 to 4 mm. Eventually the tissues between the spots yellow and turn brown, but the individual spots remain distinguishable by their darker color and zonate markings. The zonation is comparable to that developed in wildfire of tobacco and bacterial leaf spot of Delphinium. When the infections occur close together, the individual spots are small (1 mm. or less in diameter), but by coalescing they form large black or sepia-black areas. A slimy bacterial exudate is noticeable at times on the lesions. Leaf infections weaken the leaves to such an extent that defoliation results.

² CLINTON, G. P. REPORT OF THE BOTANIST. Conn. Agr. Expt. Sta. Bien. Rpt. 1907-08 (pt. 12): 870, 1909.

Entrance of the bacteria is accomplished through the stomata. (Pl. 1, B.) Sometimes the organism enters the veins from the intercellular spaces or through the water pores at the tips of the serratures, so that the disease becomes systemic. When this occurs the veins darken and the surrounding tissues yellow and deaden, thereby assuming the appearance shown in Figure 4. This effect suggests the behavior and appearance of water-pore lesions typical of black rot on cabbage, but cross inoculations between *Bacterium campestre* and the poppy organism were negative.

EFFECT ON THE STEMS

The lesions on the stems are intensely black, as are those on the leaves (fig. 5); but, unlike the leaf lesions, the stem lesions tend to elongate longitudinally. When close together they may coalesce and girdle the stems. They are confined chiefly to the cortical regions, but the girdling process is sufficiently severe to cause the smaller plants to break over.

EFFECT ON FLORAL PARTS AND PODS

All floral parts are susceptible, but the young sepals are especially so; these may be partially or entirely blackened. (Fig. 5.) The organism is able to pass through the sepals and attack the young petals beneath. When this occurs further development of the flowers is stopped.

The spots on the pods (fig. 5), like those on the leaves, are black and conspicuous, but the margins are more promi-

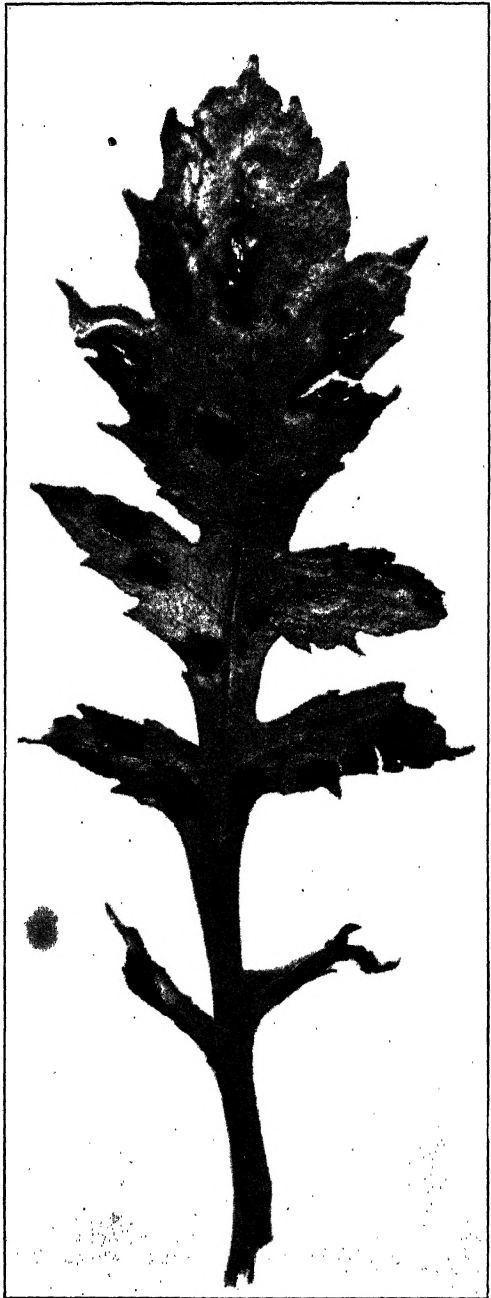


FIGURE 1.—Shirley poppy leaf showing isolated spots of infection. About twice natural size

nently water-soaked. The slimy appearance of the spots, referred to above, is more noticeable on the pods than on any other part of the plant.



FIGURE 2.—Shirley poppy leaf with numerous scattered spots of infection and large dead areas formed by coalescing spots. Natural size

GENERAL EFFECT ON THE PLANT

The general effect on the plants has a twofold economic significance. In the first place, the conspicuous black leaf spots make the plants unsightly; and secondly, many of the plants are killed. The killing is caused in part by the girdling of the stems, in part by defoliation, and in part by the toxic or mechanical effect produced by masses of the organism when the disease becomes systemic.

PREVIOUSLY REPORTED BACTERIAL DISEASES OF POPPY

The only report of bacterial blight of poppy, previous to that made by the junior writer, is that of Clinton.³ Other bacterial diseases of poppy have been reported as noted below.

Marchal and Foex⁴ in 1915 described a blight of the sepals and petals of poppy, but they did not designate the species from which they isolated a white bacterium similar to *Bacillus coli communis*. They did not make any inoculations with the organism that they isolated. The organism described in the present paper produces a yellow pigment.

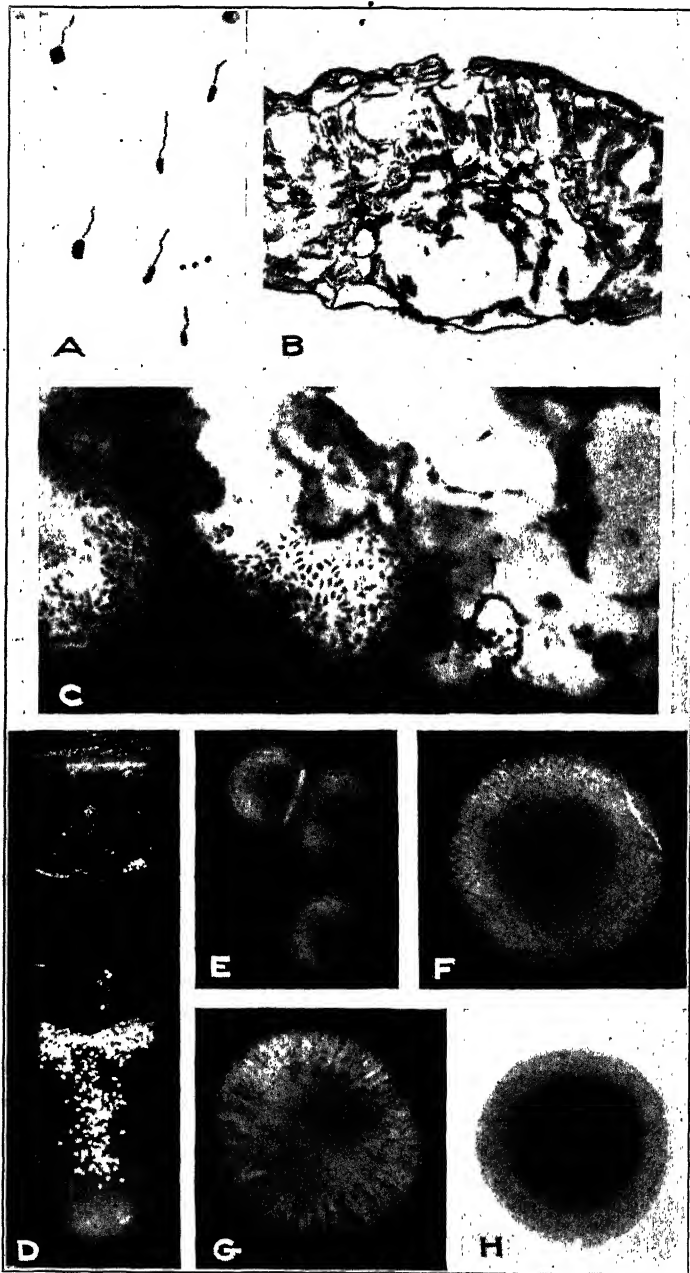
In 1918 Hutchinson⁵ reported from India a poppy disease which he considered to be due to bacteria. In describing it he stated:

"Blackening and slimy decay of the stem and leaves of the opium poppy and other varieties was found to be due to bacterial

³ CLINTON, G. P. Op. cit.

⁴ [MARCHAL, P., and FOEX, E.] RAPPORT PHYTOPATHOLOGIQUE POUR L'ANNÉE 1913. Ann. Serv. Epiphyties 2:63-64. 1915.

⁵ HUTCHINSON, C. M. AGRICULTURAL BACTERIOLOGY. India Bd. Sci. Advice Ann. Rpt. 1916-17: 57-66. 1918.



A.—*Bacterium papavericola* showing flagella. \times about 1,100.
 B.—Section of inoculated leaf showing stomatal infection. \times 200.
 C.—Section of diseased leaf showing bacteria in the tissues. \times 1,150.
 D.—Milk culture 1 month old, showing masses of tyrosin crystals.
 E-H.—Colonies on beef-infusion agar plates. \times 5. Colony E, 3 days old; colonies F, G, and H, 5 days old. Colonies E, F, and G by oblique transmitted light; colony H by direct transmitted light.



rot; a description of the disease and of the causative organism is in hand for publication." It appears that Hutchinson did not publish his data but instead turned them over to Ram Ayyar,⁶ as reported below.

In 1927 Ram Ayyar⁷ described from Pusa, India, a bacterial soft rot of garden poppy which is apparently that referred to by Hutchinson. The disease and the causal organism described by him are different from those recorded in this paper. The Indian disease found on *Papaver somniferum* at Cawnpore by Hutchinson later developed on *P. rhoeas* at Pusa, and the latter species was used in inoculation experiments by Ram Ayyar. Ram Ayyar made no reference to leaf spotting, but instead he described the disease as a soft rot of the stem and the organism as a peritrichiate, rod-forming, white or bluish-white growth on various culture media. The bacterial-blight organism of the present paper forms a yellow pigment and produces no soft rot.

ISOLATIONS AND INOCULATIONS

The organism was found abundantly in old and young lesions on both species of the poppies studied. (Pl. 1, C.) It was readily isolated from fresh material by the poured-plate method, and subcultures from single colonies were used in inoculation experiments.

On June 20, 1927, a group of Shirley poppy plants in a garden, the only ones then available, were sprayed with a suspension of the pathogene isolated on June 2 from Shirley poppy. A gentle rain which continued during the evening provided sufficient moisture so that it was not necessary to cover the plants with inoculation cages. Infections were evident on stems and leaves on June 25.

Pure-culture reisolations made on June 28 from spotted leaves on these inoculated plants were used on September 21 to inoculate plants of Shirley and Oriental poppies by spraying. These plants were kept moist in inoculation cages in the hothouse for 48 hours. The Shirley poppies used were blooming plants, whereas the Oriental



FIGURE 3.—Isolated spots of infection on Shirley poppy leaf, enlarged to show zonate character. $\times 5$

⁶ RAM AYYAR, C. S. A BACTERIAL SOFT ROT OF GARDEN POPPY. India Dept. Agr. Mem., Bact. Ser. 12: 29-33, illus. 1927.

⁷ RAM AYYAR, C. S. Op. cit.

ones were seedlings with only a rosette of leaves. All were vigorous. Successful infections were thus obtained on the leaves, buds, and seed pods of the Shirley poppy and on the leaves of the Oriental poppy. On October 7 the pathogene was reisolated from all parts of these infected plants.

Poured-plate isolations from spots on Oriental poppy leaves collected at North, Va., were also used on September 21 to inoculate both species of poppy. The same method was used as with the isolations from the Shirley poppy. Infections resulted on both species, and successful reisolations were made.

Good infections were also obtained when Shirley poppy plants in an outdoor plot were sprayed with the pathogene and kept moist under large flower pots.

The Shirley poppy appears to be more susceptible than the Oriental poppy, judging by the number of infections resulting from these series of inoculations.

THE PATHOGENE

MORPHOLOGY

Bacterium papavericola, sp. nov., is a short rod, 1 to 1.7μ long by 0.6 to 0.7μ wide, motile by means of a single polar flagellum. (Pl. 1, A.) It occurs singly, in pairs, or in short chains. Capsules occur in old agar cultures and in the rims and pellicles of bouillon cultures. No spores have been observed.

STAINING REACTIONS

The organism is decidedly Gram-negative and is not acid-fast. It stains readily with the usual bacterial stains.

CULTURAL CHARACTERS

FIGURE 4.—Systemic infection on leaf of Shirley poppy. Natural size



Unless otherwise stated, the descriptions here given are based on cultures of the Shirley strain, but they are true also for those of the Oriental strain. Slight differences in amount rather than character of growth have been disregarded.

BEEF-AGAR PLATES.⁸—Colonies are visible on the second day, and by the third day they are 2 to 3 mm. wide, mustard yellow,⁹ smooth, convex, round, finely crosshatched by transmitted light, translucent, margin entire. (Pl. 1, E.) Radiating lines are sometimes conspicuous (pl. 1, G) and at other times entirely lacking. (Pl. 1, F.) Colonies may attain a width of 7 mm. and often are slightly umbonate, the thinner margin showing as a paler yellow ring by transmitted light. (Pl. 1, H.) Secondary growths of paler color, in the form of

⁸ Unless otherwise noted, all beef media used were made with beef infusion and peptone and had a pH of 6.8 to 7.0.

⁹ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

wedges on the margin or circular areas in the body of the colony, are common in old colonies. A second type of colony sometimes appears; these are smaller, thinner, and deeper yellow. Colonies of the Oriental strain differ from those of the Shirley strain in internal markings only. They occasionally show fine concentric lines in the margins, but they are often finely granular throughout without other markings.

BEEF-AGAR SLANTS.—Growth is filiform, and scanty at first, becoming moderate but never heavy; it is translucent, smooth, finely granular or crosshatched, butyrous in young cultures, becoming viscid when old. The color, at first mustard yellow,¹⁰ becomes primuline yellow with age (2 weeks). Numerous small, circular, secondary growths of paler yellow appear in old cultures.

The growth on slant cultures of the Oriental strain is consistently deeper yellow (primuline to light cadmium) than on those of the Shirley strain.

BEEF BROTH.—Clouding is prompt, and by the second day a pale-yellow rim and often an incomplete pellicle floating unattached to the rim have formed. In undisturbed cultures the pellicle becomes heavy and may or may not connect with the rim. Numerous small rectangular crystals form in the pellicle.

Growth on beef-extract agar and broth does not differ from that on agar and bouillon made with beef infusion.

POTATO CYLINDERS.—Growth is heavy on potato cylinders, covering the whole surface of the potato and filling the water with a dense, yellow slime. The diastatic action is so strong that the cylinder gradually shrinks until almost completely submerged in the yellow bacterial growth, which may eventually take on a brown tinge. Minute brown, spherical aggregates of crystals form in abundance between the growth and the side of the tube in old cultures. Cultures of the Oriental strain are indistinguishable at first from those of the Shirley strain. Eventually the growth becomes a decidedly deeper yellow and less abundant than in the Shirley strain, and the diastatic action is less vigorous.

THAXTER'S POTATO-DEXTROSE AGAR.—Growth on slant cultures is very abundant, pale yellow, and homogeneous. Color deepens with age to primuline yellow. The Oriental strain remains paler than the Shirley one on this medium.

SYNTHETIC MEDIA.—In Cohn's solution growth may not occur, and at best it is almost imperceptible. Fermi's solution clouds weakly but develops no fluorescence. In Uschinsky's solution clouding is slow, becoming moderate but without fluorescence.

PHYSIOLOGICAL CHARACTERS

LIQUEFACTION OF GELATIN.—In beef-infusion gelatin held at 17° to 20° C. liquefaction begins in two or three days; it is saucer shaped at first, but later it becomes stratiform. It proceeds so slowly that after two weeks only one-half

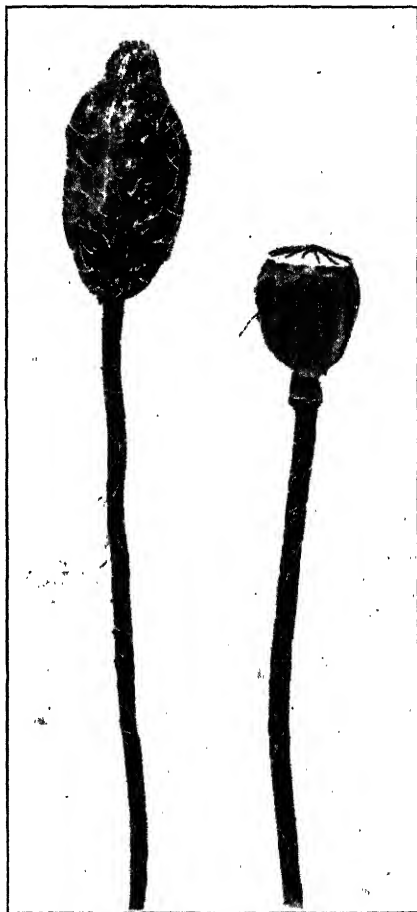


FIGURE 5.—Infections on bud, pod, and floral stalk of Shirley poppy. Natural size

¹⁰ RIDGWAY, R. Op. cit.

is liquefied. Liquefaction is not complete even when the cultures are held for six weeks. The liquefied portion is clear, with a yellow pellicle and abundant sediment.

LIQUEFACTION OF BLOOD SERUM.—Liquefaction, which is preceded by clearing of the medium, begins by the third day; clearing and liquefaction proceed so slowly that in three weeks only one-half of the medium is liquefied.

FERMENTATION OF SUGARS.—Various sugars and alcohols were tested for fermentation. On beef-extract agar slant cultures with bromcresol purple as an indicator, acid was evident on the third day from saccharose, dextrose, and galactose. The yellow color progressed slowly to the bottom of the tubes. On the fourth day an alkaline reaction appeared in the upper end of all these cultures, and by the fifteenth day the agar was alkaline throughout in all. At no time was there any evidence of acid formation from levulose, lactose, maltose, glycerin, or mannit in this agar. When phenol red was used as an indicator in the same agar and with the same sugars, an alkaline reaction was evident on the second or third day, and in 10 days the whole of each culture was alkaline.

The organism was tested on the same carbohydrates in synthetic agar made according to the formula in the Manual of Methods¹¹ with bromcresol purple as indicator. There was no trace of alkali at any time in these cultures. An acid reaction was evident in saccharose and dextrose on the second day, on the fifth day in glycerin, and on the third or fourth day in the others. Within three weeks all the cultures were acid throughout.

No gas was formed from these sugars and alcohols in fermentation-tube cultures containing 1 per cent peptone and 2 per cent of the sugar. Clouding was confined to the open ends of the tubes.

HYDROLYSIS OF STARCH.—The starch of steamed-potato cylinders is so completely destroyed that the shrunken cylinders are almost or completely submerged in the mass of bacterial slime. On starch-agar plates streaked with the organism, an area 25 mm. wide is cleared beyond the growth within seven days. The diastatic action of the Oriental strain is decidedly less vigorous. The potato cylinders are never completely submerged. On starch-agar plates the clear area after growth for seven days is only 5 to 8 mm. wide.

REDUCTION OF NITRATE.—No nitrate reduction was evident in nitrate-broth cultures, when 4, 7, and 10 days old, by using the starch-potassium-iodide sulphuric-acid test. However, a weak but decided nitrite reaction was obtained at all these ages by the α -naphthalamine sulphanilic-acid test.

REACTIONS IN MILK.—Separation begins on the second day with a layer of whey 1 to 3 mm. deep above a fluid curd. In litmus milk there is a slight reddening on the third day, followed immediately by reduction of the litmus, which is completed in 4 to 10 days. Color begins to return 3 or 4 days later. Peptonization is evident on the third or fourth day; clearing proceeds slowly downward in bands and is completed in two weeks. In cultures 1 month old the curd is a coarsely flocculent, fluid, translucent mass occupying the lower half of the culture, with a clear whey above, holding numerous masses of tyrosine crystals. (Fig. 4.)

Reduction of methylene blue in milk begins on the third day and is completed on the fourth day. Coagulation, peptonization, and the formation of tyrosine crystals proceed as in litmus milk. Old cultures (1 month) are orange buff in color.

PRODUCTION OF AMMONIA.—Ammonia production is strong, as indicated by tests made with strips of filter paper wet with Nessler's solution and inserted in beef-broth cultures 8 and 10 days old.

PRODUCTION OF HYDROGEN SULPHIDE.—Lead-acetate paper tests for the presence of hydrogen sulphide in beef broth gave on the third day a distinct positive reaction which became strong by the sixth day.

PRODUCTION OF INDOL.—No indol was formed in Dunham's solution within 15 days. *Bacillus coli communis* used as a check gave the usual positive result.

TOLERATION OF SODIUM CHLORIDE.—The organism grows readily in beef broth containing 1.2 or 3 per cent sodium chloride. Growth is retarded by 4 per cent and inhibited by 5 per cent.

RELATION TO FREE OXYGEN.—In agar stabs the best growth occurs at and near the surface; no growth occurs in the bottom of the stabs, or in stabs which have been covered immediately by 10 c. c. of agar. There is no growth in the lower part of shake agar cultures. Growth is confined to the open end of fermentation tubes.

OPTIMUM PH FOR GROWTH.—In a series of beef broths ranging from pH 4.4 to 9.6, clouding occurred within 24 hours in pH 6.1 to 7.7, but it was heaviest between pH 6.9 and 7.3. By the second day all were clouded between pH 5.6 and 8.3. No growth took place beyond these limits.

RESISTANCE TO DESICCATION.—Sterile cover glasses wet with a loop from 24-hour-old cultures were placed in sterile Petri dishes and kept dry in the dark. At intervals covers were dropped into beef broth. After drying for four months and at intervals before, the bacteria clouded the bouillon promptly.

In herbarium specimens the bacteria were alive after drying for 20 months. On plates from these dry leaves colonies came up promptly and appeared as vigorous as those from fresh material; they were proved infectious by inoculation on Shirley poppy leaves.

TEMPERATURE RELATIONS.—The thermal death point is 52° C. Best growth occurs between 25° and 30°. The maximum temperature for growth is about 35°.

TECHNICAL DESCRIPTION

***Bacterium papavericola*, sp. nov.**

A short rod, 1 to 1.7 μ long by 0.6 to 0.7 μ wide, motile by means of a single polar flagellum, occurring singly, in pairs, or in short chains. It is capsulate but forms no spores; is Gram-negative and not acid-fast; forms round, bright-yellow colonies on beef agar; clouds bouillon and forms a rim and a pellicle; diastatic action strong; liquefies gelatin rather slowly; forms acid without gas from dextrose, saccharose, galactose, levulose, lactose, maltose, glycerin, and mannit; reduces nitrates; causes weak coagulation and complete peptonization in litmus milk with reduction of litmus and the formation of tyrosine crystals; produces ammonia and hydrogen sulphide but no indol; is aerobic; grows best in a pH of 6.9 to 7.6; withstands drying for 4 months on cover glasses and for 20 months or longer in herbarium specimens; optimum temperature is between 25° and 30° C.; causes black spots on all aboveground parts of the Shirley poppy and on leaves and pods of the Oriental poppy.

SUMMARY

Bacterial blight of poppy is a disease which attacks Shirley and Oriental poppies. It produces unsightly black spots on leaves, stems, buds, and seed pods; it spreads rapidly and when severe may kill the plants.

The disease has been found in Virginia and Connecticut. The organism causing it has been isolated and successfully inoculated into Shirley and Oriental poppies. *Bacterium papavericola* is presented as the name for the pathogene. A description of the cultural and physiological behavior of *Bact. papavericola* is given.

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SPECIES OF THE NEMATODE GENUS *STRONGYLOIDES* PARASITIC IN DOMESTIC SWINE¹

By BENJAMIN SCHWARTZ, *Senior Zoologist*, and JOSEPH E. ALICATA, *Junior Zoologist, Zoological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The object of this paper is to clear up the existing confusion and uncertainty with reference to the specific identity of the nematodes of the genus *Strongyloides* parasitic in domestic swine. The name *Strongyloides suis* has been used by certain workers to designate a distinct zoological species, and by others it has been used merely as a convenient host designation for *Strongyloides* from swine. Probably the majority of helminthologists who have expressed an opinion on this subject have regarded *S. suis* as a synonym or as a probable synonym of *S. papillosus* of sheep. The investigations reported in this paper, together with a study of the literature relating to *Strongyloides* of swine, have shown that two species of this nematode genus have been reported from these host animals. One species, which is the form involved in an extensive account of the pathogenicity of *Strongyloides* to young pigs by Reisinger (14),² is considered in this paper as *S. suis*, and the other species, which presumably is the form which many helminthologists have considered identical with *S. papillosus*, is regarded as new.

REVIEW OF LITERATURE

Reisinger (14), Sandground (15), Baylis (1), and other workers who have studied *Strongyloides* have overlooked the fact that Pagenstecher (9) recorded *Strongyloides* from pigs, presumably for the first time, and that his observations antedated those of Grassi (3) and Lutz (7). Lutz has erroneously been credited with being the first to report *Strongyloides* from swine. Pagenstecher (9) records small, slender worms, consisting entirely of female specimens, from the small intestines of pigs. He recognized these worms as related to *Anguillula* (synonym, pro parte, of *Strongyloides*) and described their morphology in considerable detail, listing all the salient generic characters of the genus *Strongyloides*. Pagenstecher says that the ovary is short in young specimens and that it increases in size as the worms grow older, finally becoming twisted in loops around the intestine, anteriorly and posteriorly, a character which has been figured and described with reference to *Strongyloides* of swine by later workers.

Grassi (3) reports that animals other than man harbor *Anguillula intestinalis* (as used by Grassi this name is a synonym of *Strongyloides stercoralis*, parasitic in man); he records these parasites from swine, rabbits, and weasels, and he also refers to their occurrence in cows, chickens, cats, sparrows, and mice, and notes their absence in several species of amphibians and reptiles which he examined. He states

¹ Received for publication May 10, 1929; issued January, 1930.

² Reference is made by number (italic) to "Literature cited," p. 22.

that the forms from the different hosts differ in certain respects, especially in size, and expresses the opinion that they belong to the same genus.

The same author (4) used the name *Rhabdonema longus* for the form from sheep and reiterates the opinion that the same species also occurs in the rabbit, the weasel, and the pig. He says that the parasitic female is 7 mm. long and notes that in the free-living generation of *R. longus* the females outnumber the males to the extent of 1,000 to 1. He also notes that the free-living females of this species often die without having copulated.

Lutz (7), apparently unaware of previous records of the occurrence of Strongyloides in pigs, reports observations on these nematodes from swine, based on a study of the parasitic females which he obtained on necropsy from a pig in Brazil, as well as observations on the free-living males and females which he cultured in the feces obtained from the same host animal. Lutz says that he recognized the similarity of these forms to those of *Anguillula stercoralis*, from man, as described by Perroncito (10), noting, however, certain differences in size. He states that the parasitic females from the swine are about 1 cm. long and that the free-living males and females, which he obtained from fecal cultures in approximately equal proportions, are twice the size of those cultured from human feces. He also notes that the eggs of the pig Strongyloides hatch outside of the body, whereas those of the human form hatch before they are eliminated with the feces.

Von Linstow (6) uses the name *Strongyloides suis* for the first time and erroneously credits Lutz with being the author of this name. Von Linstow not only proposed a new name, but at the same time that he proposed it he also relegated it to the synonymy, as he lists *S. suis* as a synonym of *S. longus* (Grassi and Segré) and gives the following hosts for this species: *Ovis aries*, *Sus scrofa*, *Lepus cuniculus*, *Foetorius vulgaris*, and *Mus decumanus*. Aside from listing the hosts of *S. suis* and stating that it is 6 mm. long, he gives no description. *S. longus* (Grassi and Segré) is regarded at the present time as a synonym of *S. papillosus*.

Perroncito (10) reports Strongyloides from swine, especially from young pigs, and gives an account of the pathogenicity of these parasites. His paper contains no data with reference to the morphology of the worms, which he does not name specifically.

Ransom (13) reports for the first time, the occurrence of Strongyloides in pigs in the United States. He makes the following statement with reference to the specific identity of Strongyloides from American swine:

The parasite of the pig, which has been identified as *Strongyloides longus* (= *S. papillosus*) by European observers, is probably *Strongyloides suis*. This species has been found a number of times in pigs at the Bureau of Animal Industry Experiment Station, Bethesda, Md.

Railliet (11) lists *Strongyloides suis* (Leuck.) from swine. The present writers have been unable to trace any reference to the specific name *suis* used by Leuckart in combination with any synonyms of the genus Strongyloides.

Reisinger (14), in an extensive paper on the pathogenicity of Strongyloides to young pigs, gives very valuable clinical data and figures and describes a species of Strongyloides for which he uses the

names *S. suis* and *S. longus* interchangeably. As will be shown later, the morphology of the worms as figured and described by Reisinger differs in several important respects from that of the worms which have been collected from pigs in Bethesda, Md., and studied by the writers.

Marotel (8) describes a chronic enteritis in pigs due to *Strongyloides suis*. His paper is not available to the writers. The abstracts of this paper which have been consulted contain little information in regard to the morphology of the worms, the parasitic female being described only as from 3 to 4 mm. long by 30μ to 40μ wide.

Fiebiger (2) regards *Strongyloides* from pigs as identical with *S. longus* of sheep and rabbits. His figure of this species from pigs agrees with Reisinger's figure of *S. suis*.

Hall (5) recognizes *Strongyloides suis* as a valid species, listing *S. longus* (pro parte) as its synonym, and noting for the first time that the forms found in the eastern part of the United States do not agree with the description of *S. suis* as given by European helminthologists.

Sandground (15) questions the validity of *Strongyloides suis* and states that he examined several specimens (parasitic females) of *Strongyloides* from swine obtained from the helminthological collection of the Bureau of Animal Industry, and found that the ovary is twisted around the intestine, as in *S. papillosus*. He says:

The only possible distinction that could be made between these specimens and *S. papillosus* would be on the basis of a more acutely pointed tail in *S. suis*; but as this character is very variable, the distinction between *S. suis* and *S. papillosus* on morphological grounds is uncertain.

Sandground's statement with reference to the variability of the shape of the tail in the two species might be interpreted as implying that the forms from pigs and sheep overlap with regard to this morphological feature. As will be shown later, such overlapping has not been observed by the writers. On the contrary, the shape of the tail is remarkably constant for the sheep form as well as for the form from pigs, the difference between the two being very distinct and striking.

Baylis (1) says:

S. suis von Linstow, 1905, a form originally recorded by Lutz in 1885 from the pig, appears to be a very doubtful species, and is probably identical with *S. papillosus*.

There are several additional references to *Strongyloides* in swine which, in the main, are repetitions of opinions expressed by other workers and supported by little, if any, original observation or critical examination of published data.

It is evident from this summary of the literature with reference to *Strongyloides* in swine that the views expressed by Grassi with reference to the specific identity of the sheep and swine forms have largely prevailed and that a number of later workers who have accepted these views have not supported them with morphological evidence based on original observations. A conspicuous exception is Ransom (13), who was familiar with the forms from the two hosts and did not consider them identical. It should also be noted that Hall (5) observed the discrepancies between descriptions by European investigators of the morphology of *Strongyloides* from swine and the forms from swine occurring in the eastern part of the United States

with which he was familiar, and that he also recognized the fact that *S. longus* is a synonym of *S. suis* only so far as the former has reference to forms from pigs.

OCCURRENCE OF STRONGYLOIDES IN AMERICAN SWINE

Aside from the brief references made by Ransom (13), Hall (5), and Sandground (15) to the occurrence of Strongyloides in American swine, there appear to be no published data concerning these parasites of domestic swine in the United States. In view of the recorded pathogenicity of Strongyloides, especially to young pigs, based on a study of clinical symptoms of infested animals by Perroncito (10), Reisinger (14), and Marotel (8), it appears important to determine whether a presumably important pathogenic parasite of young pigs has been overlooked by most American parasitologists.

As a preliminary step to an experimental study of the pathogenicity of Strongyloides for pigs, it appeared essential to determine: (1) Whether one or more than one species of Strongyloides has been recorded from these host animals; and (2) whether the view shared by certain helminthologists with reference to the probable identity of *S. papillosus* and *S. suis* is supported by morphological and other evidence.

Examinations of pigs at the Bureau of Animal Industry Experiment Station, Bethesda, Md., for the presence of Strongyloides were begun by the senior author in 1927. Cultures of feces from young pigs, from a few weeks to a few months old, revealed, after about 24 hours, the presence of rhabditiform larvae, and after 48 hours or longer the presence of filariform larvae and of free-living males and females, the number of males present being very few as compared with the number of females. In the course of post-mortem examinations of young pigs, which were killed at various times in connection with certain experiments, parasitic females belonging to the genus Strongyloides, were obtained from the small intestine. An examination of these specimens showed that they differed in several important respects from *S.*

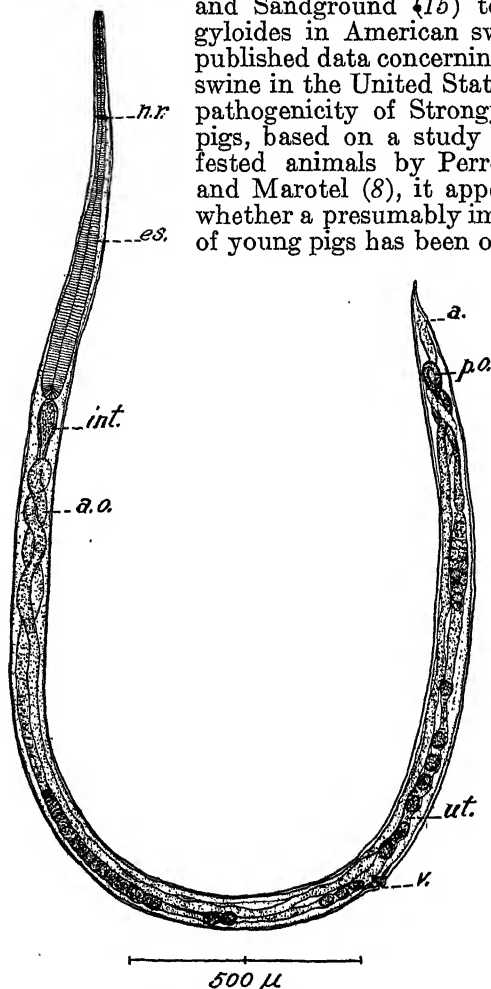


FIGURE 1.—*Strongyloides ransomi*; parasitic female. a., anus; a. o., anterior ovary; int., intestine; n. r., nerve ring; es., esophagus; p. o., posterior ovary; ut., uterus; v., vulva

iform larvae and of free-living males and females, the number of males present being very few as compared with the number of females. In the course of post-mortem examinations of young pigs, which were killed at various times in connection with certain experiments, parasitic females belonging to the genus Strongyloides, were obtained from the small intestine. An examination of these specimens showed that they differed in several important respects from *S.*

papillosus of sheep and also from the figures and descriptions of *S. suis* published by investigators in Europe.

In the course of necropsies on three young pigs at Moultrie, Ga., by E. B. Cram and E. W. Nighbert, of the Zoological Division, several slender nematodes were collected from the small intestine and forwarded to the senior author for determination. Unfortunately these worms were poorly preserved, so that when they were examined no morphological details could be made out, but the shape of the tail resembled that in figures of *Strongyloides* from pigs published by European workers. The female tail which is figured and described by Reisinger and figured in Fiebiger's textbook on parasitology is highly attenuated, in contrast to the conical tail with a more or less blunt tip found in all specimens of *Strongyloides* collected from swine at Bethesda. A recent restudy of the latter forms by the writers and a comparison of these forms with those from sheep showed that the swine form represents a species distinct from the sheep form and also distinct from the pig form as figured and described from Europe. The name *S. ransomi* is proposed for the American species, the name *S. suis* being retained for the time being for the forms with acutely pointed tails. The latter species probably occurs in this country, as forms resembling it were collected from swine at Moultrie, Ga.

***Strongyloides ransomi*, new species.**

Specific diagnosis: *Strongyloides*:

Parasitic females (fig. 1). From 3.33 to 4.49 mm. long and from 54μ to 62μ wide. Body long, filiform, and of nearly equal thickness from the region of the base of the esophagus to the region of the posterior ovarian loops. Beginning at about the region of the base of the esophagus, the body narrows gradually toward the anterior end, the head being attenuated to a diameter of about 15μ . Posteriorly the body diminishes in size backward. The diameter of the body in the region of the anus is from 23μ to 31μ . In the region of the posterior ovarian loops the body narrows considerably and becomes gradually reduced behind the anus, terminating in a distinctly tapering, conical tail, the tip of which is more or less blunt. The esophagus is from 605μ to 883μ long by 47μ wide. The diameter of the body in the region of the base of the esophagus is from 47μ to 54μ wide. The anus is located at a distance of from 53μ to 83μ from the posterior end. The vulva is a transverse slit, with salient lips, situated posterior to the middle of the body but considerably anterior to the last third of the body, at a distance of from 1.1 to 1.6 mm. from the tip of the tail. The ovary is twisted in loops anteriorly and is less constantly looped posteriorly. The posterior ovary is not uncommonly bent in a hairpin fashion, with a tendency to cross over and form a loop. (Fig. 2.) The eggs (fig. 3) are ellipsoidal, thin shelled, from 45μ to 55μ long, 26μ to 35μ wide, and contain an embryo at the time that they are eliminated with the feces.

Table 1 gives a summary of the measurements of eight specimens of parasitic females.

TABLE 1.—Measurements of eight specimens of parasitic females of *Strongyloides ransomi*

Size		Esophagus		Location of vulva		Length of tail
Length	Width	Length	Width	From anterior end	From posterior end	
Mm.	μ	μ	μ	Mm.	Mm.	μ
4.495	62	853	47	2.821	1.674	79
3.72	54	780	47	2.325	1.395	67
4.03	62	605	47	2.968	1.162	68
4.65	62	853	47	2.39	1.66	83
3.332	62	713	47	1.922	1.41	71
3.952	62	822	47	2.821	1.131	67
3.689	62	883	47	2.124	1.565	53
3.67	62	775	47	2.245	1.425	79

Free-living generation:

Males (figs. 4 and 5). From 868μ to 899μ long and 54μ wide. Body of nearly equal diameter except in the tail region. Posterior to the anus the body becomes considerably narrowed, tapering gradually and terminating in a relatively long, slender tail; anteriorly the body narrows gradually, commencing in the region of the base of the esophagus. The mouth leads into a short pharynx; the esophagus is from 132μ to 140μ long and 23μ wide in the posterior bulbous portion. Spicules from 26μ to 29μ long, shaped like curved blades and having a knob-like handle. The gubernaculum is from 18μ to 18.7μ in maxi-

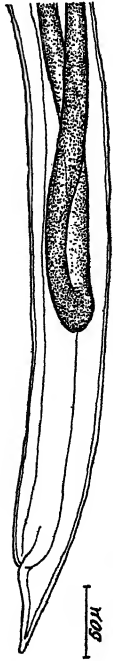


FIGURE 2.—*Strongyloides ransomi*, posterior end of parasitic female

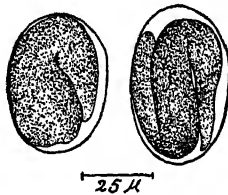


FIGURE 3.—Eggs of *Strongyloides ransomi*



FIGURE 4.—*Strongyloides ransomi*, anterior end of male. *es.*, intestine; *n. r.*, nerve ring; *int.*, esophagus; *t.*, testis

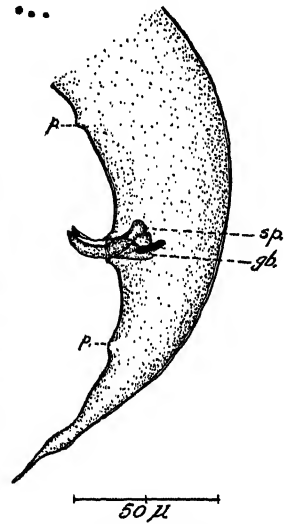


FIGURE 5.—*Strongyloides ransomi*, posterior end of male. *gb.*, gubernaculum; *p.*, papilla; *sp.*, spicule

mum diameter, and 9.4μ in minimum diameter. One preanal and one postanal papilla, ventral in position, approximately equidistant from the anus. The tail is from 83μ to 90μ long.

Females (fig. 6). From 1 to 1.1 mm. long and 62μ wide in the region of the vulva. The body tapers very gradually anteriorly and narrows to a diameter of about 13μ to 15μ in the head region; the diameter of the body in the region of the esophagus is from 43μ to 47μ . Posteriorly the body tapers more abruptly than anteriorly, becoming considerably narrowed in the region of the rectum and tapering gradually to a slender tail. The diameter of the body in the region of the anus is about 23μ . The mouth leads into a short pharynx; the esophagus is from 124μ to 155μ long and 23μ wide in the posterior bulb. The vulva has salient lips and is located near the middle of the body. In young forms comparatively few ovarian eggs are present in the uterus, but in gravid females numerous shelled eggs are contained in the anterior and posterior uteri. After oviposition is completed the uterus shrinks in size and the ovaries appear to be degenerated. The tail is from 150μ to 158μ long.

Rhabditiform larvae:

The rhabditiform larvae (figs. 7 and 8) are from about 280μ to somewhat over 400μ long and about 20μ in maximum width, the smaller forms being newly hatched. The pharynx is short, from 5μ to 6μ long. The esophagus is from about 70μ to nearly 90μ long. The genital primordium, which is about 15μ to 18μ long, is located approximately in the region corresponding to that of the middle of the intestine. The tail is tapering, about 55μ long.

Filariform larvae:

These larvae (fig. 9) are from 504μ to 635μ long and from 15μ to 19μ wide in the region of the base of the esophagus. The esophagus is from 240μ to 310μ

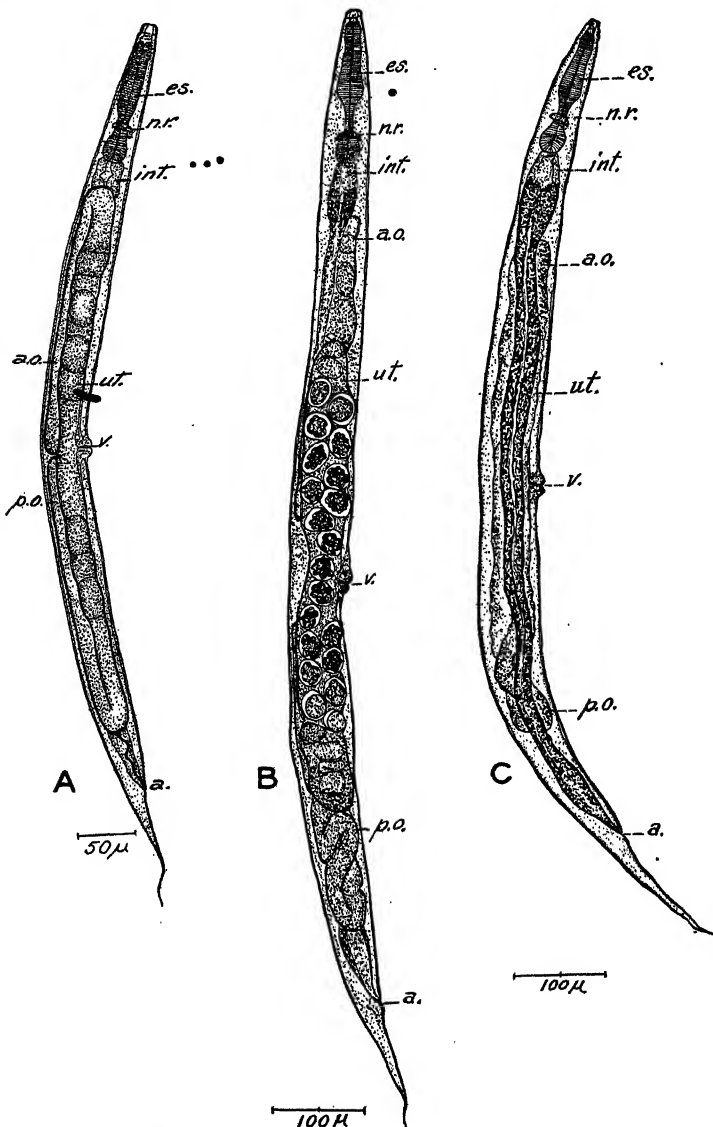


FIGURE 6.—*Strongyloides ransomi*: A, Young female; B, gravid female; C, female after oviposition. a., anus; a. o., anterior ovary; int., intestine; n. r., nerve ring; es., esophagus; p. o., posterior ovary; ut., uterus; v., vulva

long and from 7.5μ to 11μ wide. The tail is tapering from 60μ to 90μ long. The genital primordium is located at a distance of from 168μ to 225μ from the tip of the tail, approximately in the region corresponding to that of the middle of the intestine.

Host.—*Sus scrofa domestica*.

Location.—Small intestine (embedded in mucosa). Free-living generation and rhabditiform and filariform larvae may be cultured in feces of infested hosts.

Type locality.—Bethesda, Md.

Type specimen of parasitic female.—Bureau of Animal Industry helminthological collection, United States National Museum No. 28779.

Paratypes of parasitic female.—Bureau of Animal Industry helminthological collection, United States National Museum No. 28780.

Type specimens of free-living male and female.—Bureau of Animal Industry helminthological collection, United States National Museum No. 28777.

Paratypes of free-living male and female.—Bureau of Animal Industry helminthological collection, United States National Museum No. 28778.

This species is dedicated to the memory of the late Brayton Howard Ransom, who reported, for the first time, *Strongyloides* from swine in the United States and who carried out important experimental work on skin penetration by *S. papillosus* and on the experimental transmission of this species to rabbits.

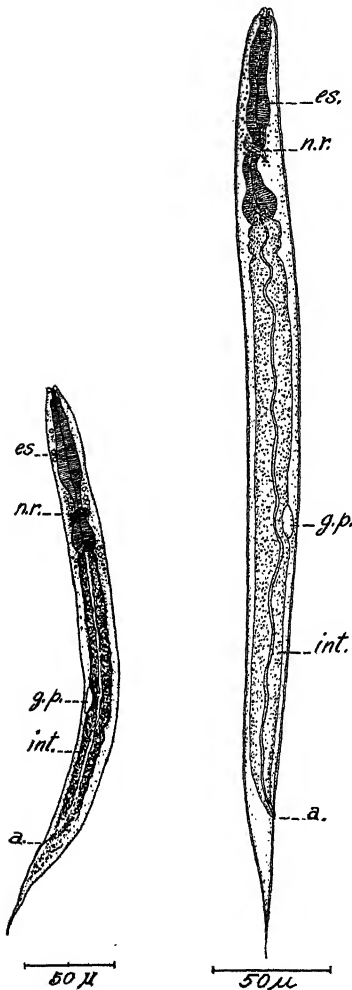


FIGURE 7.—Newly hatched rhabditiform larva of *Strongyloides ransomi*. a., anus; g. p., genital primordium; int., intestine; n. r., nerve ring; es., esophagus

FIGURE 8.—Fully grown rhabditiform larva of *Strongyloides ransomi*. a., anus; g. p., genital primordium; int., intestine; n. r., nerve ring; es., esophagus

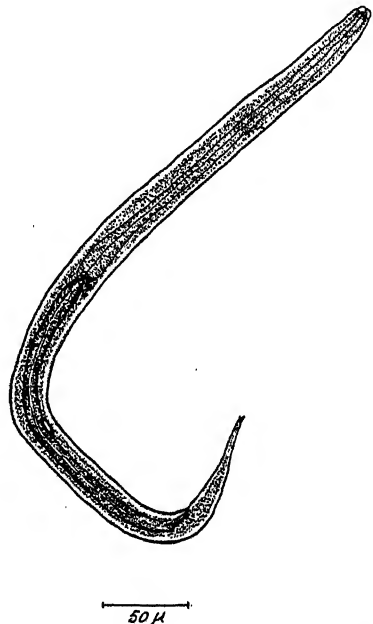


FIGURE 9.—Filariform larva of *Strongyloides ransomi*

COMPARISON OF STRONGYLOIDES RANSOMI WITH *S. PAPILLOSUS*

In a comparison of the parasitic females of *Strongyloides ransomi* with *S. papillosus*, the shape of the tail presents a striking difference. That of *S. papillosus* (fig. 10) is of fingerlike shape and markedly

blunt, whereas that of *S. ransomi* (fig. 11) is cone-shaped and tapers gradually, its tip being more or less bluntly rounded. Among numerous specimens of parasitic females from sheep and swine which were examined, the shape of the tail was found to be constant for each species, and the variations were not found to overlap. Aside from

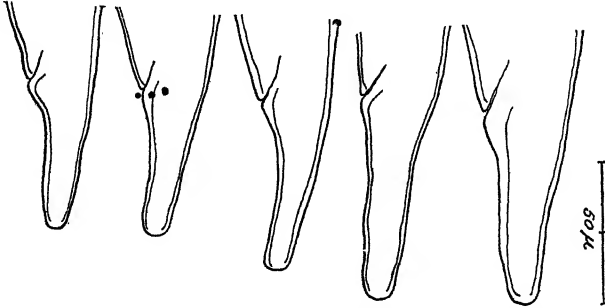


FIGURE 10.—Tails of parasitic females of *Strongyloides papillosus*

this conspicuous difference, no other highly significant morphological differences have been found in comparing the forms from the two hosts. As regards the size of the eggs, those of *S. ransomi*, obtained from fresh pig feces, showed a range of from 45μ to 55μ long by 26μ

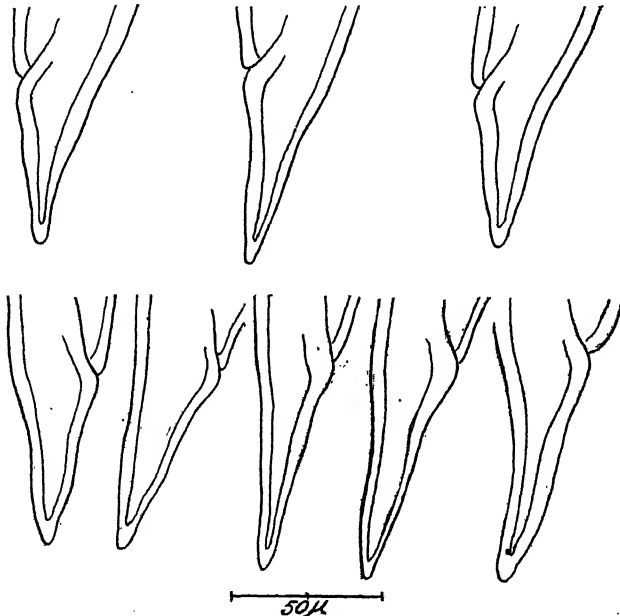


FIGURE 11.—Tails of parasitic females of *Strongyloides ransomi*

to 35μ wide, whereas those obtained from fresh sheep feces showed a range of from 48μ to 67.5μ long by from 30μ to 34μ wide. It is of interest to note that of 10 measured eggs of *S. ransomi* 8 were less than 50μ long and 2 were between 50μ and 55μ long, whereas 12 of 20 eggs of *S. papillosus* ranged from 60μ to 67.5μ in length, and only 1

egg was less than 50μ long, the remaining 7 eggs being between 52μ and 56μ long. In a general way the eggs of *S. papillosus* are longer and more elongated than those of *S. ransomi*.

It is also noteworthy that whereas cultures of feces from swine infested with *Strongyloides ransomi* have yielded in all cases free-living males, cultures of feces from sheep infested with *S. papillosus* have thus far failed to show the presence of males. Ransom (12) carried out a number of experiments on the transmission of *S. papillosus* from sheep to rabbits, and he apparently failed to find males in his cultures. In 1911 he gives a fairly detailed description of the free-living female and states that the male is undescribed. Baylis (1) states that the male of *S. papillosus* is apparently rare. He gives the first description of the free-living male of *S. papillosus* based on one specimen as follows: 750μ long by 35μ wide, tail 70μ long, spicules 35μ long. While the size measurements are consistently smaller than those recorded in this paper for *S. ransomi*, it is significant that the spicules of *S. papillosus* are considerably longer than those of *S. ransomi*, according to Baylis's measurements of those of the former species. The spicules of four specimens of *S. ransomi* showed the following lengths: 26μ , 28μ , 28μ , 29μ . It is to be regretted that Baylis does not give the host from the feces of which the male was cultured. One can not assume with absolute certainty that the host in question was a sheep, since Baylis lists the following hosts for *S. papillosus*: Sheep, goat, ox, pig, and rabbit.

As regards the filariform larvae, 6 specimens of *Strongyloides ransomi* showed the following range in size: 503μ long, 16μ wide; 635μ long, 18μ wide; 519μ long, 18μ wide; 542μ long, 18μ wide; 519μ long, 16μ wide; 496μ long, 15μ wide. Seven filariform larvae of *S. papillosus* showed the following range in size: 651μ long, 17μ wide; 666μ long, 15μ wide; 681μ long, 15μ wide; 710μ long, 15μ wide; 651μ long, 15μ wide; 574μ long, 15μ wide; 666μ long, 15μ wide. While these measurements overlap, it is important to note that the filariform larvae of *S. papillosus* are considerably longer than those of *S. ransomi*, the maximum size of the latter being close to the minimum size of the former. The size of the esophagus of the filariform larvae in the two species shows a variation of from 230μ to 279μ in length by from 11μ to 15μ in width for *S. papillosus*, and a variation of from 240μ to 310μ in length by from 7.5μ to 11μ in width for *S. ransomi*. While these measurements overlap, it is important to note that the individual measurements of the esophagus for the two species show differences as follows: *S. papillosus*: 240μ by 11μ , 271μ by 11μ , 263μ by 11μ , 279μ by 15μ , 255μ by 11μ , 230μ by 11μ , and 279μ by 11μ ; *S. ransomi*: 255μ by 7.5μ , 310μ by 7.5μ , 240μ by 7.5μ , 263μ by 11μ , 263μ by 7.5μ , and 255μ by 7.5μ . Aside from the fact that the base of the esophagus of the filariform larvae of *S. papillosus* is wider in most specimens than that of *S. ransomi*, the ratio of the length of the esophagus as compared to the total length of the body is considerably larger in the latter species. The location of the genital primordium with reference to its distance from the anus in the two species is as follows: *S. ransomi*: 121μ , 125μ , 109μ , 118μ , 105μ ; *S. papillosus*: 147μ , 152μ , 170μ , 170μ , 170μ , 151μ , 165μ .

Aside from the morphological differences between *S. ransomi* and *S. papillosus* which have been noted, there also appears to be a biological difference between these two species, so far as concerns their ability to develop in the rabbit. As first shown by Ransom (12),

S. papillosus can develop to fertile maturity in rabbits. In order to determine whether there are any differences between *S. ransomi* and *S. papillosus* with regard to the degree of adaptability to various abnormal hosts, the following experiments were performed:

Filariform larvae of *S. ransomi* were fed to a cat, a guinea pig, a rat, two rabbits, and a chick. These animals were held under observation for varying periods ranging from about three weeks to two months, during which interval the feces were examined for eggs and were cultured for the presence of larvae, with negative results in all cases. One rabbit in this series was fed filariform larvae on April 3, 1929; the animal died on April 24 and a careful examination failed to reveal the presence of *Strongyloides* in the intestine. The second rabbit was fed filariform larvae on May 7, 1929, and was killed on July 6; during this interval no eggs were detected in the feces and on post-mortem examination no *Strongyloides* were found in the intestine.

Filariform larvae of *S. papillosus* were fed to two rabbits, two guinea pigs, and a chick. One rabbit, which was fed larvae on April 13 and 15, failed to show eggs in the feces up to May 10, on which day it was chloroformed. At necropsy 54 specimens of *Strongyloides*, most of which were not gravid, were collected from the intestine. The second rabbit was fed filariform larvae of *S. papillosus* on May 21, 1929; eggs were first noted in the feces of this rabbit on June 17 and continued to be eliminated with the feces up to July 8, during which interval several fecal examinations were made. Since July 11 the feces of this rabbit have been consistently negative for eggs, and cultures of the feces have failed to show the presence of larvae. The guinea pigs and chick were kept under observation more than six weeks, during which interval they showed no eggs in the feces; these animals showed no *Strongyloides* at necropsy.

It is evident from these experiments that *S. papillosus* can develop to fertile maturity in rabbits, while *S. ransomi* is apparently not adapted to this host.

STRONGYLOIDES SUIS

The status of the name *Strongyloides suis* still remains to be considered. As used by Von Linstow (6), this name has little, if any, zoological standing, as it is not accompanied by a description. Von Linstow apparently had in mind the species of *Strongyloides* from swine described by Lutz (7) which is described, however, without reference to specific details, the characters mentioned in the description being, with the exception of the size of the females, generic. For the time being, the name *S. suis* may be retained for the form described and figured by Reisinger (14) which, so far as can be judged from his illustration, appears to be identical with that figured by Fiebiger (2) from swine. Reisinger's species may be characterized as follows:

Parasitic females (fig. 12). From 4 to 6 mm. long by 80 μ wide, attenuated at the anterior end. Esophagus one-fifth of the total length. Uterus contains

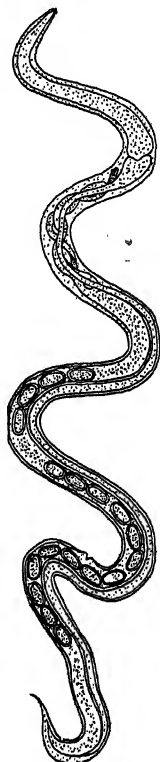


FIGURE 12.—*Strongyloides suis*. (From Reisinger, 1915)

20 to 30 eggs. Vulva near the posterior fourth of the body. Eggs 60μ long by 40μ wide containing an embryo three and one-half to four times as long as the egg. Tail long and acutely pointed.

Reisinger failed to obtain the free-living generation in cultures. The forms which he figures as possibly representing the free-living males and females were obtained from feces in hogs and are unquestionably free-living nematodes.

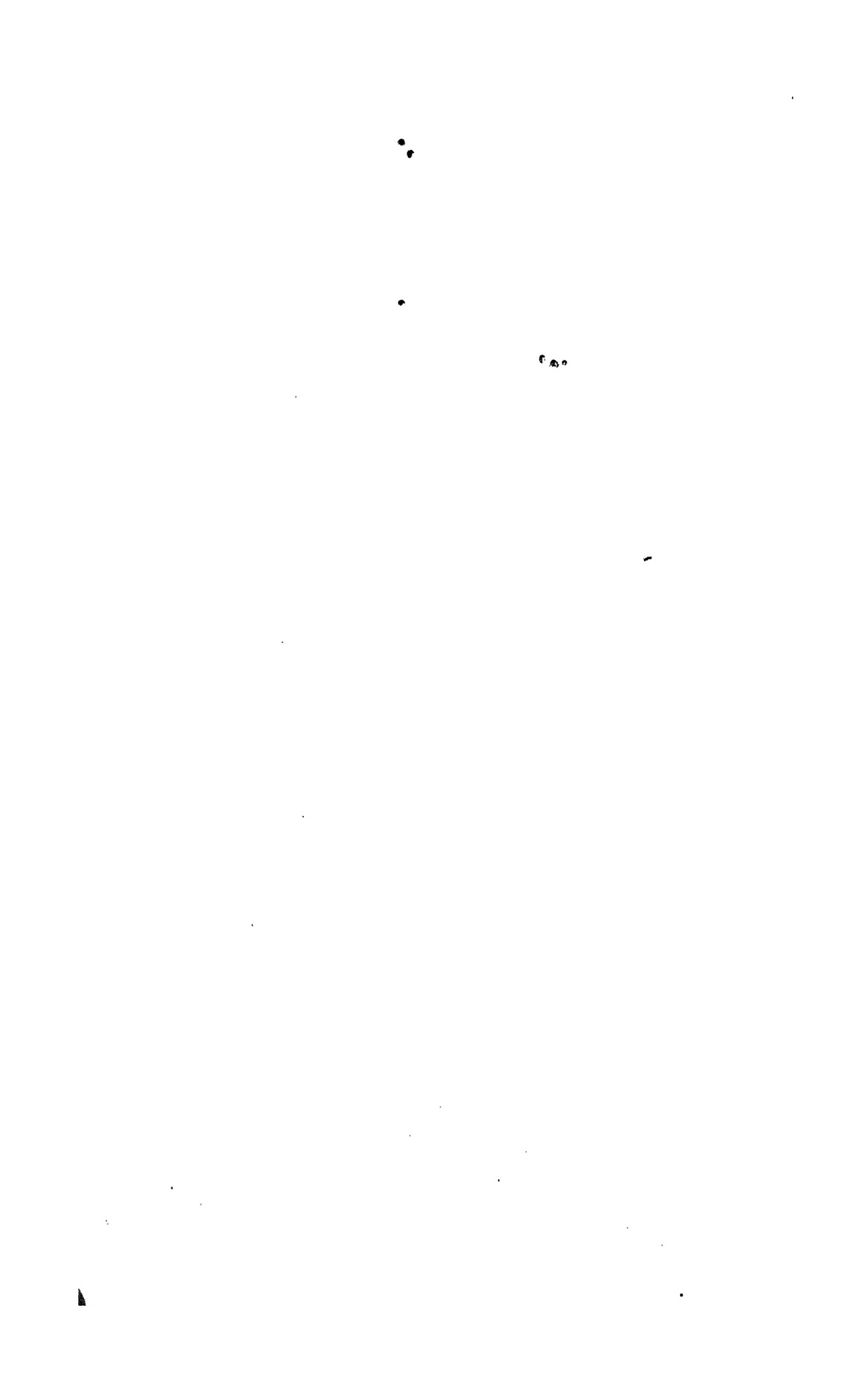
The striking character of Reisinger's form is the acutely pointed tail, which so far as is known, and if accurately figured, is unique in the genus *Strongyloides*. The position of the vulva, approximately in the beginning of the posterior fourth of the body, represents another difference between this form and *S. ransomi*, in which latter the vulva in the parasitic female is located at some distance anterior to the beginning of the posterior third of the body.

In view of the fact that Fiebiger's figure of *Strongyloides* from pigs, designated by him as *S. longus*, corresponds to that of Reisinger, at least so far as the shape of the female tail is concerned, and that forms resembling these worms were collected from pigs in Georgia, in which case it is to be noted that symptoms of digestive disturbance were observed and ascribed to these parasites, the recognition of these forms as a species distinct from *S. ransomi* is warranted. For the time being the name *S. suis* which has been used by many helminthologists for the species from swine may be retained for the long-tailed European forms, largely as a matter of convenience. The retention of this name is also justified from the standpoint of nomenclature.

LITERATURE CITED

- (1) BAYLIS, H. A.
1929. A MANUAL OF HELMINTHOLOGY, MEDICAL AND VETERINARY. 303 p., illus. London.
- (2) FIEBIGER, J.
1923. TIERISCHE PARASITEN DER HAUS- UND NUTZTIERE, SOWIE DES MENSCHEN. EIN LEHR-UND HANDBUCH MIT BESTIMMUNGSTABELLEN FÜR TIERÄRZTE, ÄRZTE UND STUDIERENDE. Aufl. 2, enl. and improved, 439 p., illus. Wien and Leipzig.
- (3) GRASSI, G. B.
1878. L'ANGUILLULA INTESTINALIS. NOTA PREVENTIVA. Gaz. Med. Ital.-Lombardia (7) 5: 471-474.
- (4) ———
1885. CONTRIBUZIONE ALLO STUDIO DELLA NOSTRA FAUNA. Accad. Gioenia Sci. Nat. Catania Atti (3) 18: 241-252.
- (5) HALL, M. C.
[1924]. WORM PARASITES OF DOMESTICATED ANIMALS. PARASITES OF SWINE. 160 p., illus. [Chicago.]
- (6) LINSTOW, [O. F. B.] VON
1905. STRONGYLOIDES FÜLLEBORNI N. SP. Centbl. Bakt. [etc.] (I. Originale) 38: 532-534, illus.
- (7) LUTZ, A.
1885. ÜBER EINE RHABDONEMA ART DES SCHWEINES, SOWIE ÜBER DEN BEFUND DER RHABDONEMA STRONGYLOIDES (ANGUILLULA INTESTINALIS UND STERCORALIS) BEIM MENSCHEN IN BRASILIEN. Centbl. Klin. Med. 6: [385]-390.
- (8) MAROTEL, G.
1920. SUR UNE MALADIE PARASITAIRE NOUVELLE, L'ANGUILLULOSE PORCINE. Bul. Soc. Sci. Vét. [Lyon] 1920: 110. [Original not seen, abstract in Rev. Gén. Méd. Vét. 30: 395-396. 1921.; also Rev. Vét. [Toulouse] (3 s., t. 2) 73: 412-413. 1921.]

- (9) PAGENSTECHE, H. A.
1865. DIE TRICHINEN. NACH VERSUCHEN IM AUFTRAGE DES GROSSHERZOGLICH BADISCHEN HANDELSMINISTERIUMS AUSGEFÜHRT AM ZOOLOGISCHEN INSTITUTE IN HEIDELBERG . . . 116 p., illus. Leipzig.
- (10) PERRONCITO, E.
1906. LE SOSTANZE TOSSICHE PRODOTTE DAI PARASSITI ANIMALI. Ann. Accad. d'Agr. Torino (1905) 48: [273]-278.
- (11) RAILLIET, A.
1914-15. L'EMPLOI DES MÉDICAMENTS DANS LE TRAITEMENT DES MALADIES CAUSÉES PAR DES NÉMATODES. Rec. Méd. Vét. 91: 490-513.
- (12) RANSOM, B. H.
1907. NOTES ON PARASITIC NEMATODES, INCLUDING DESCRIPTIONS OF NEW GENERA AND SPECIES, AND OBSERVATIONS ON LIFE HISTORIES. U. S. Dept. Agr., Bur. Anim. Indus. Circ. 116, 7 p.
- (13) ———
1911. THE NEMATODES PARASITIC IN THE ALIMENTARY TRACT OF CATTLE, SHEEP, AND OTHER RUMINANTS. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 127, 132 p., illus.
- (14) REISINGER, L.
1915. ÜBER DAS VORKOMMEN UND DIE PATHOLOGISCHE BEDEUTUNG VON STRONGYLOIDES LONGUS BEIM SCHWEIN. Wiener Tierärztl. Monatsschr. 2: [209]-239, illus.
- (15) SANDGRÖUND, J. H.
1925. SPECIATION AND SPECIFICITY IN THE NEMATODE GENUS STRONGYLOIDES. Jour. Parasitol. 12: [59]-80, illus.



SOME EFFECTS OF SEED TREATMENT ON THE GERMINATION AND SUBSEQUENT GROWTH OF WHEAT¹

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INTRODUCTION

Where winter wheats can be successfully grown they outyield spring wheats. For the years 1910 to 1927, inclusive, the difference in yield for the country as a whole was 15.7 per cent in favor of winter wheat (17).² When winterkilling occurs, it is sometimes possible to reseed with winter wheat early in the spring or during the late winter. The question as to how late winter wheat may be spring-sown and yet mature normally is of vital interest to the wheat grower under such conditions.

It is a well-recognized fact that true winter wheats sown at spring planting time will not head until late fall or the following summer. This condition has been termed "vegetative dormancy," which, although similar, is hardly analogous to the dormancy of seeds of woody plants.

A recent case of vegetative dormancy was noted in Peru. "The winter wheats, Hybrid 128, Fortyfold, and Turkey failed to produce culms when planted in early June, which is the beginning of the fall season. They reacted much like winter wheats planted in the spring in the States. Spring varieties produced a normal growth."³

Several causes have been assigned for the failure of winter wheat to mature normally when spring seeded. The growth habit is a heritable character. Aside from this, there are certain environmental factors which seem necessary for the expression of the character of winter habit. Exposure to freezing in the field, favorable light relations, and nutritional factors have been suggested as indispensable to normal growth. Investigators have shown that these factors do affect the growth periods.

REVIEW OF LITERATURE

Many workers have studied the effects of seed treatment on the germination of seeds. Comparatively few, however, have studied the growth of plants produced from treated seeds past the very earliest growth stages. The literature here cited is chiefly that which deals with subsequent growth.

Tincker (16) found that soaking oat grains in water accelerated seedling growth and concluded that a definite correlation existed between the vigor of germination and the rate of subsequent growth. Jones and Tincker (9) state that seed stimulation as an aid to rapid growth of seedlings was found to affect yields of oats.

Kiessling (11), using a large number of chemicals, was able to increase the germination of wheat, oats, and barley. Gleisberg (6)

¹ Received for publication Apr. 16, 1929; issued January, 1930.

² Reference is made by number (italic) to "Literature cited," p. 35.

³ E. V. Abbott, Plant Pathologist, Estacion Experimental Agricola, Lima, Peru. Correspondence.

increased the vegetative growth of radish 364 per cent by treating the seeds with a solution containing 15 per cent magnesium chloride and 15 per cent magnesium sulphate. Seed treated in distilled water yielded more than twice that of the control. Silbert (15) reported increased yields and somewhat earlier maturity from Canada field peas, soybeans and buckwheat when treated with a solution of copper sulphate. Popoff (14) concluded that it was possible to increase the yields of crops 30 to 50 per cent by treating the seeds with solutions of chemicals before planting.

Denny (3) studied the effects of numerous organic chemicals on freshly harvested potatoes. With many of these he was able to overcome the dormant period and force sprouting.

Several investigators have studied the effects of different temperatures on seeds. Munerati (13) concluded that fresh seed exposed to low temperatures at the germination period made a higher and a more rapid germination. Fawcett (4) found that exposure of weed seeds to low temperatures with intermittent thawing increased the germination and shortened the dormant period. Coffman (2) states that small grains will germinate at the temperature of melting ice.

Jensen (8) noted little or no effect on subsequent vegetative growth after soaking wheat in water and freezing it before planting. Howard (7), working with 65 species of woody plants, found that freezing was the best treatment for forcing growth. Kidd and West (10) have made a comprehensive review of the literature on physiological predetermination. Of the work mentioned, that of Gassner is of the most interest. He found that exposure of the seeds of several winter cereals to low temperatures during germination or early seedling growth assures flowering the first year. The earlier in the development of the plant the treatment was given the more pronounced the results obtained. Similar treatment with summer annuals did not appreciably affect the time at which culm formation took place.

The effects of light on the vegetative periods of plants have been studied by several investigators. Garner and Allard (5) state that in any region the relative lengths of day and night constitute one of the controlling factors in plant development. Klages (12) concluded that there was no material difference between winter and spring wheat growth habits when the plants were grown in the greenhouse. Wanser (18) concluded that the "photoperiod" or favorable length of day was the key to the distinction between winter and spring wheats.

MATERIALS AND METHODS

The purpose of the investigation herein reported was to determine the possibility of using chemical or freezing treatments of germinating wheat seed as a means of breaking or modifying the vegetative dormancy of the resulting plants.

The work as carried out required nine hundred and sixty-nine 8-foot rows in the field trials. Germination counts were made on 340 lots of treated wheat. Root and sprout lengths in millimeters were taken on 3,400 seedlings grown in a standard germinator. Germination and seedling vigor were used as indices of the effects of seed treatments and formed the bases for determining the most desirable concentrations of solutions for field trials. Both germination and field trials were run in duplicate.

The following 16 varieties of wheat were used: White Winter, Triplet, Turkey, Blackhull, Kharkof, Ridit, Fortyfold, Hybrid 128, Oro, White Odessa, Kanred, Hybrid 63, Hybrid 143, Hard Federation, Marquis, and Baart. Pure seed from nursery breeding trials was used in this experiment. It is realized that hand-threshed pure-line seed of each variety would have been better. Seed of this type, however, was not available. All heads formed were carefully checked

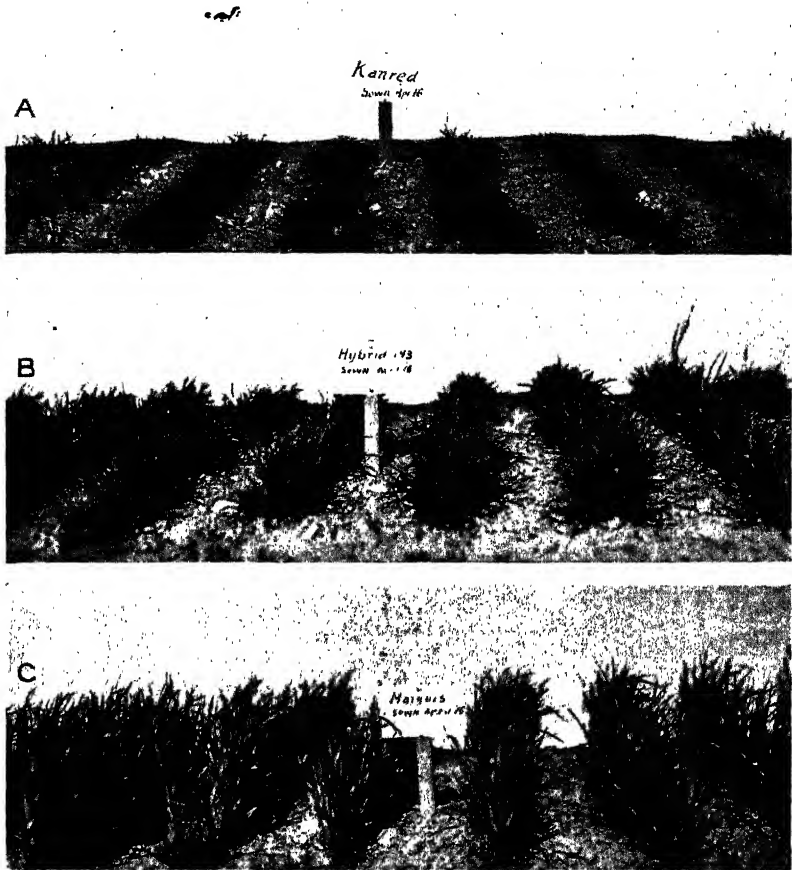


FIGURE 1.—Early growth habits of typical winter, semiwinter, and spring wheats, sown April 16 and photographed 34 days after spring seeding: A, Kanred winter wheat; B, Hybrid 143 semiwinter wheat; C, Marquis spring wheat

for varietal characteristics and were field sown in the spring of 1928 to determine the habits of growth.

The first 11 varieties are of the winter type. Hybrids 143 and 63 are semiwinter and the last three are spring wheats. Figure 1 shows the early growth habits of these three types of wheat. Ridit is a hard red winter selection from the cross Turkey \times Florence, made at Pullman, Wash. Oro is a Turkey selection made at Moro, Oreg. The other varieties are described in United States Department of Agriculture Bulletin 1074 (1).

The chemicals, the length of treatment, and the concentrations used are listed below.

Trichloroethylene, 3 hours in a 6 per cent solution.

Ethylene chloride, 3 hours in a 1 per cent solution.

Ethylene chlorhydrin, 3 hours in an 8 per cent solution.

Ethyl bromide, 3 hours in a 6 per cent solution.

Ammonium thiocyanate, 3 hours in a 3 per cent solution.

Potassium thiocyanate, 3 hours in a 1 per cent solution.

Carbon bisulphide, 2 hours in water and 1 hour in a 3 per cent solution.

These chemicals were used by Denny (3) in hastening the sprouting of dormant potato tubers. With the exception of the thiocyanates, they are liquids in the commercial form. It is recognized that trichloroethylene, ethylene chloride, ethyl bromide, and carbon disulphide are insoluble or only slightly soluble, so true solutions are not formed with water in some cases. The mixture of chemical and water, however, will be referred to as a solution.

The solutions were made and the treatments given in earthen bowls at a temperature of approximately 20° C. The grain was weighed in 10-gm. lots and placed in cheesecloth bags for treating. To avoid differences in concentration of solutions all varieties were treated



FIGURE 2.—General view of the field nursery in which wheats were grown after seed treatment with various chemicals; the winter wheats are in the foreground, semiwinter at the lower center, and the spring wheats immediately behind the semiwinter

simultaneously in the same solutions. After submersion, the bags were allowed to drain one and one-half hours, after which the seed was field planted or transferred to germination blotters.

The lots frozen were first soaked in water 1 hour and allowed to remain in the germinator 20 hours before removal to the cold chamber. Moisture tests showed that the moisture content was about 18 per cent. This procedure allowed the seeds to begin germination before being frozen. Freezing was done in a dairy refrigeration room at a constant temperature of -12° C. Freezing was followed by chemical treatment. The treated material for all lots was planted in the field April 16, 1927, in 8-foot rows at the rate of 2 bushels per acre and at a depth of 2 inches.

Four series of trials were run in duplicate. New and aged seeds were used. Freezing was used alone and in combination with chemical treatment. Both wet and dry checks were included. Germination and seedling-growth studies were made as an index of the effect of seed treatment for determining the concentrations of solutions to be used. Root growth, germination, and sprout growth were recorded. For the field-seeded material the percentages of survival and the growth habits were determined. Dates of first heading, full heading, and ripening were observed. The numbers of heads of winter wheat which ripened by September 1 were recorded.

DISCUSSION AND RESULTS

EFFECT OF TREATMENTS ON NUMBER OF HEADS PRODUCED

Table 1 shows the number of heads produced by winter wheats in the first series of treatments when the chemicals as previously listed were used. Figure 2 shows a general view of the field nursery. The relative growth of the three types of wheat is clearly shown. In this series the grain was soaked one hour in running water at a temperature of 17° C. This soaking gave a moisture content of approximately 18 per cent. The grain was drained 10 minutes and placed in the solutions for treatment. After treatment the lots were immediately seeded in the field. Two checks were used, one a dry, untreated lot and the other soaked in water for a period corresponding to the length of the chemical treatments.

TABLE 1.—Number of heads produced by spring-sown winter wheats after seed treatment with various chemicals

[Duplicate rows 8 feet in length]

SERIES 1

Chemical	Number of heads produced by wheat varieties indicated								Total
	Turkey	Black-hull	Khar-kof	Forty-fold	Hybrid 128	Oro	Ridit	White Odessa	
Potassium thiocyanate.....	14	5	4	2	0	5	0	0	30
Ammonium thiocyanate.....	0	0	7	0	0	1	0	0	8
Trichloroethylene.....	2	6	7	2	0	3	0	0	20
Ethylene chlorhydrin.....	2	6	1	3	0	2	0	0	14
Ethylene chloride.....	1	11	4	1	0	0	0	0	17
Ethyl bromide.....	1	6	10	0	0	4	0	0	21
Carbon disulphide.....	3	3	4	0	0	1	0	0	11
Wet check.....	4	6	4	1	0	0	0	0	15
Dry check.....	0	0	7	2	0	2	0	0	11
Total.....	27	43	48	11	0	18	0	0	147

SERIES 2

Potassium thiocyanate.....	1	9	5	0	0	1	0	0	16
Ammonium thiocyanate.....	0	5	1	0	0	0	0	0	6
Trichloroethylene.....	9	10	6	0	0	0	0	0	25
Ethylene chlorhydrin.....	5	7	11	1	0	2	0	0	26
Ethylene chloride.....	4	11	9	1	0	3	0	0	28
Ethyl bromide.....	1	24	5	1	0	3	0	0	34
Carbon disulphide.....	5	10	6	2	0	1	0	0	24
Wet check.....	4	9	9	2	0	1	0	0	25
Dry check.....	3	7	9	4	0	0	0	0	23
Total.....	32	92	61	11	0	11	0	0	207

The heads of winter wheat which appeared in 1927 were harvested, and as a check for mixtures they were field-seeded in the spring of 1928. The varieties Kanred, White Winter, and Triplet contained true spring-wheat strains which had the typical morphological characters of the parent varieties. Because of the presence of these spring strains the results for the above varieties are not given in Table 1. The spring types will be grown in the nursery in 1929 for further study.

It may be noted (Table 1) that potassium thiocyanate produced more heads than other treatments, ethyl bromide was second and trichloroethylene third. Ammonium thiocyanate produced the fewest. The variety Kharkof produced the most heads, and Blackhull slightly fewer. Hybrid 128, Redit, and White Odessa failed to produce heads. True spring and semiwinter wheats were fully headed at the normal dates.

In Table 1 data are also given for series 2. This series is a duplication of series 1 except that before being planted the treated grain was placed in a standard germinator for 20 hours at a temperature of 21° C. Of the chemicals used, ethyl bromide produced the largest number of heads, with ethylene chloride closely following. With the exception of ammonium thiocyanate, potassium thiocyanate, and carbon disulphide, the chemical treatments produced an increase in the number of heads. These increases are not large enough to justify definite conclusions.

As in series 1 so in series 2 the grain treated with ammonium thiocyanate produced fewest heads. It may be noted that the treatments, based on number of heads produced, do not rank as in the first series. With the exception of the thiocyanate treatments, each produced more heads in series 2 than in series 1. Taking the total number of heads for each series, it will be seen that series 2 produced 40 per cent more heads than series 1. More heads were produced by all varieties heading in series 2 than in series 1 with the exception of Fortyfold which produced the same number in both series and Oro which produced a greater number of heads in series 1. The only difference between these series was the incubation of the treated grain used in the second series.

To get the general effect of the chemicals and the reaction of the varieties the figures for both series have been combined, although the treatments in each case are not the same. Combining the results of both series, the treatments rank as follows on a basis of heads produced: Ethyl bromide, 55; potassium thiocyanate, 46; trichloroethylene, 45; ethylene chloride, 45; wet check, 40; ethylene chlorhydrin 40; carbon disulphide, 35; dry check, 34; ammonium thiocyanate, 14.

Similarly, when heads produced by each variety are grouped for both series, the varieties rank as follows: Blackhull, 135; Kharkof, 109; Turkey, 59; Oro, 29; Fortyfold, 22; White Odessa, 0; Hybrid 128, 0; Redit, 0.

Since the number of heads produced was the only variable one noted, this is used to represent the effects of the treatments. The heads produced by a variety are assumed to represent the relative degree to which that variety is vegetatively dormant. Redit, White Odessa, and Hybrid 128 may be said to more dormant than Blackhull.

Freezing of seeds in a germinated condition, combined with chemical treatments, did not break the vegetative dormancy of the resulting plants. Table 2 gives a summary of data for White Winter and

Kanred, new and aged seed being used. The figures represent percentage of stand and heads produced. It should be remembered that from these two varieties spring strains were derived when ripened heads were used as seed the following year. In this series the thiocyanates show a decided reduction in stand and heads produced. Trichloroethylene-treated seeds show an increase in number of heads over the average of the checks, but not higher than the check frozen dry, which produced the best stand and the largest number of heads.

TABLE 2.—*The effect upon stand and head production of soaking wheat seed in water 1 hour, allowing it to germinate 20 hours, freezing it 12 days at -12° C., and treating it with various chemicals*

[Duplicate rows 8 feet in length]

Treatment	Results with Kanred, using—				Results with White Winter, using—			
	Previous crop seed		1923 crop seed		Previous crop seed		1925 crop seed	
	Stand ¹	Heads produced ²	Stand	Heads produced	Stand	Heads produced	Stand	Heads produced
	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>	<i>Number</i>
Potassium thiocyanate.....	50	1	15	1	75	2	40	0
Ammonium thiocyanate.....	30	1	20	0	75	0	10	0
Trichloroethylene.....	90	8	50	2	100	8	75	2
Ethylene chlorhydrin.....	75	4	75	0	100	9	75	0
Ethylene chloride.....	90	2	50	2	100	5	80	0
Ethyl bromide.....	75	7	85	0	90	5	56	0
Carbon disulphide.....	85	3	75	0	100	1	80	0
Check, frozen wet.....	90	8	50	0	100	3	50	0
Check, frozen dry.....	100	5	75	3	100	12	100	3
Unfrozen check.....	100	7	100	1	100	1	100	2
Average.....	73.5	—	59.6	—	94.0	—	66.6	—
Total.....	—	51	—	9	—	46	—	7

¹ 100 per cent stand is 100 plants.

² Heads produced by Sept. 1.

Freezing 12 days at -12° C. did not entirely kill seeds of either variety of wheat. Frozen dry, both varieties germinated normally or nearly so. Frozen wet, from 10 to 50 per cent of the germs were killed. Kanred, in other trials gave a lower germination than Marquis after 12 days of freezing of soaked seed. Allowing frozen grain to thaw for 12 hours at any time in the 12-day freezing period resulted in a complete killing of every variety frozen. This would indicate that the length of the freezing period was less important than the occurrence of intermittent thawing.

A marked reduction in vitality of seed is indicated by the lower percentage of stand and number of heads produced by aged seed. (Table 2.) The amount of the reduction of germination and heading was not directly proportional to the age of the seed. Kanred produced a higher percentage of heads per plant than did White Winter.

EFFECT OF TREATMENTS ON GERMINATION OF SEEDS AND ON GROWTH OF ROOTS AND SPROUTS

Tables 3 to 5, inclusive, show data on germination, root growth, and sprout growth of grain treated with chemicals and allowed to germinate in a standard germinator at a temperature of 21° C. Germination counts were made at 48 and 96 hour periods after incubation, and root and sprout growth were measured at 96 and 144 hour periods.

TABLE 3.—*The effect of chemical treatments of the seed on the germination of four varieties of wheat*

[Hours after incubation in a standard germinator at 21° C.]

Treatment	Percentage germination of—							
	Kanred after—		White Winter after—		Marquis after—		Hybrid 143 after—	
	48 hours	96 hours	48 hours	96 hours	48 hours	96 hours	48 hours	96 hours
Potassium thiocyanate.....	95	100	92	100	100	100	93	100
Ammonium thiocyanate.....	51	62	37	38	72	87	58	59
Trichloroethylene.....	98	100	91	100	100	100	100	100
Ethyl bromide.....	93	100	19	56	97	100	97	100
Ethylene chloride.....	98	100	92	100	97	100	99	100
Carbon disulphide.....	97	100	89	100	99	100	96	100
Average.....	88.7	93.7	70.0	82.5	94.2	97.8	90.5	93.2
Check (no treatment).....	97	100	93	100	100	100	96	100

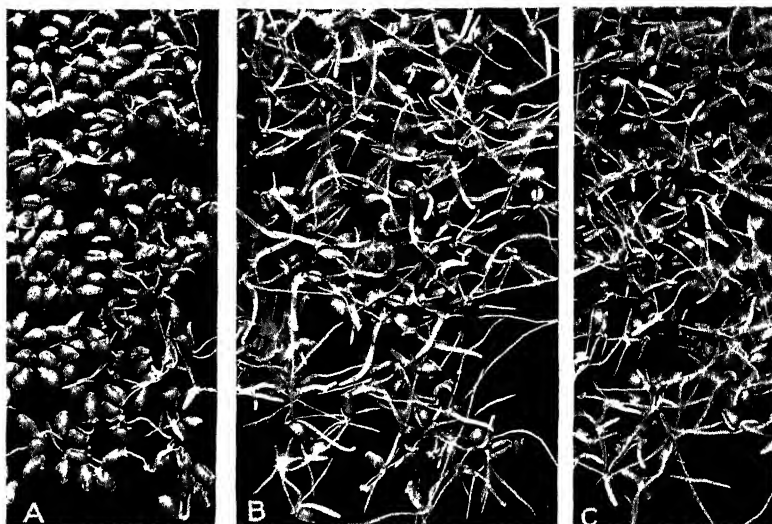


FIGURE 3.—Growth of winter-wheat seedlings in a standard germinator at a temperature of 21° C. 72 hours after treatment with ammonium thiocyanate (A), and trichloroethylene (C), compared with the untreated control (B)

Table 3 shows that the germination of the four varieties studied was rather uniform except for the marked reduction in germination of White Winter by ethyl bromide. Ammonium thiocyanate markedly reduced germination in all varieties.

Root growth (Table 4) was more variable for varieties and treatments than germination or sprout growth. Treatments of Marquis and Hybrid 133 increased root growth, but in general this was not true for Kanred and White Winter. Treatment with ammonium thiocyanate reduced root growth considerably below that of the check in Kanred and White Winter.

The data for sprout growth are given in Table 5. Ammonium thiocyanate reduced sprout growth of all varieties. No sprouts from treated seed were as vigorous as the checks. Kanred showed better sprout vigor than other varieties.

The variations in the effects of different chemicals are small except those of ammonium thiocyanate and ethyl bromide. Figure 3 shows the effects of ammonium thiocyanate and trichloroethylene on seedlings of White Winter wheat.

TABLE 4.—*The effect of chemical treatments of the seed on the root growth of four varieties of wheat*

[Hours after incubation in a standard germinator at 21° C.]

Treatment	Length in millimeters of roots of—							
	Kanred after—		White Winter after—		Marquis after—		Hybrid 143 after—	
	96 hours	144 hours	96 hours	144 hours	96 hours	144 hours	96 hours	144 hours
Potassium thiocyanate.....	46	52	37	52	44	64	35	51
Ammonium thiocyanate.....	32	37	17	25	39	51	28	39
Trichloroethylene.....	41	56	33	46	41	59	45	53
Ethyl bromide.....	41	50	26	46	37	50	47	56
Ethylene chloride.....	48	54	34	50	46	63	46	52
Carbon disulphide.....	40	54	35	53	43	58	44	59
Check (no treatment).....	43	56	41	56	15	37	15	34
Average.....	41.3	50.5	30.3	45.3	41.7	57.5	40.8	51.7

TABLE 5.—*The effect of chemical treatments of the seed on the sprout growth of four varieties of wheat*

[Hours after incubation in a standard germinator at 21° C.]

Treatment	Length in millimeters of sprouts of—							
	Kanred after—		White Winter after—		Marquis after—		Hybrid 143 after—	
	96 hours	144 hours	96 hours	144 hours	96 hours	144 hours	96 hours	144 hours
Potassium thiocyanate.....	21	37	10	19	17	37	9	22
Ammonium thiocyanate.....	11	24	4	10	9	22	5	11
Trichloroethylene.....	23	45	8	24	14	41	11	25
Ethyl bromide.....	23	43	7	22	13	34	13	30
Ethylene chloride.....	20	41	9	28	20	45	12	31
Carbon disulphide.....	22	40	11	30	12	47	12	25
Check (no treatment).....	22	43	14	31	21	45	15	35
Average.....	20	38.3	8.2	22.2	14.2	37.7	10.3	24

RELATION OF TIME OF PLANTING TO HEADING

Date of seeding is an important factor in determining the vegetative growth curve of winter wheats. An individual reaction of varieties to time of planting is noted when heading and subsequent maturity are considered.

To obtain the relation of the date of spring planting to heading, five varieties of wheat were sown at weekly intervals from January to May in 1926, 1927, and 1928. Two of the varieties, Marquis and Federation, are of spring-growth habit, and three varieties, Kharkof, Hybrid 128, and White Winter, are of winter habit. The latter variety was not seeded in 1926.

In each season the early seedings gave normal growth in all varieties. Later seedings, however, gave varied results except with Marquis, which headed normally for every date of seeding. The last dates of seeding used in these trials are given for the variety Marquis in Table 6.

TABLE 6.—*Latest date of seeding for normal heading of various varieties of wheat*

[Rows 3 feet in length]

Variety	Habit of growth	Latest date of sowing which permitted normal heading in—		
		1926	1927	1928
Marquis.....	Spring.....	¹ May 16	¹ May 23	¹ May 26
Federation.....	do.....	Apr. 26	May 23	May 12
Kharkof.....	Winter.....	Jan. 31	Mar. 5	Mar. 17
Hybrid 128.....	do.....	Jan. 31	Feb. 26	Mar. 10
White Winter.....	do.....		Mar. 19	Mar. 17

¹ Last date sown.

These results show that the heading of Hybrid 128 is greatly influenced by time of spring seeding. In the order of the effect of time of planting on heading, the varieties may be listed as follows: Hybrid 128, Kharkof, White Winter, Federation, and Marquis. The effect of season on the heading of spring-planted winter wheats was shown. The 1927 and 1928 seasons were approximately a month later than the 1926 season. Kharkof headed when planted 33 days later in 1927 and 46 days later in 1928 than for the last date of planting for heading in 1926. Four-year trials at Moro, Oreg., gave similar results.

SUMMARY

The effects of various seed treatments on the germination of wheat seed and on the growth of the plants subsequently produced has been studied. Briefly, none of the treatments used proved to be either highly stimulative to growth or a modifier of growth habits of winter, semiwinter and spring wheats.

Ammonium thiocyanate was decidedly toxic as a seed treatment, the stands being reduced and the vigor lessened even though heads were produced. In the germination trials, ammonium thiocyanate reduced germination and the growth of sprouts; it also reduced the length of roots in Kanred and White Winter wheat. Ethyl bromide was slightly toxic to root and sprout growth of White Winter wheat and reduced the germination of this variety markedly as compared with its effects on other varieties.

A difference in time of heading was noted between wet and dry checks. Those soaked in water were from one to three days earlier than those not so treated. The incubation of chemically treated seeds in the germinator for 20 hours before seeding increased the number of heads of spring-planted winter wheats.

Germinating wheat grains survived 12 days of constant freezing at -12° C. Freezing the germinating grains did not break the vegetative dormant period of winter-wheat plants subsequently produced. Alternate freezing and thawing was more detrimental to seedling growth than continuous freezing.

Marquis, a nonwinter hardy spring wheat, survived the low temperatures of the freezing trials much better than did Kanred, a fairly hardy winter wheat. Germination after exposure to low temperature did not indicate the comparative winter hardiness of these two varieties.

Winter wheats showed an individual reaction to time of planting when subsequent growth was considered. Kharkof matured when sown later than the last date of planting for normal heading of Hybrid 128. For late seasons the critical date of planting for heading is correspondingly later.

LITERATURE CITED

- (1) CLARK, J. A., MARTIN, J. H., and BALL, C. R.
1922. CLASSIFICATION OF AMERICAN WHEAT VARIETIES. U. S. Dept. Agr. Bul. 1074, 238 p., illus.
- (2) COFFMAN, F. A.
1923. THE MINIMUM TEMPERATURE OF GERMINATION OF SEEDS. Jour. Amer. Soc. Agron. 15: 257-270.
- (3) DENNY, F. E.
1926. HASTENING THE SPROUTING OF DORMANT POTATO TUBERS. Amer. Jour. Bot. 13: 118-125, illus.
- (4) FAWCETT, H. S.
1908. THE VIABILITY OF WEED SEEDS UNDER DIFFERENT CONDITIONS OF TREATMENT, AND A STUDY OF THEIR DORMANT PERIODS. Iowa Acad. Sci. 15: 25-45.
- (5) GARNER, W. W., and ALLARD, H. A.
1920. EFFECT OF RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus.
- (6) GLEISBERG, W.
1924. SAATGUTSTIMULIERUNG, EIN NEUER WEG ZU HÖHEREN ERTRÄGEN. Deut. Obst. u. Gemüsebau Ztg. 70 (4): 28-29.
- (7) HOWARD, W. L.
1915. AN EXPERIMENTAL STUDY OF THE REST PERIOD IN PLANTS. THE SUMMER REST PERIOD OF BULBS AND HERBACEOUS PERENNIALS. SECOND REPORT. Missouri Agr. Expt. Sta. Research Bul. 15, 25 p., illus.
- (8) JENSEN, I. J.
1925. WINTER WHEAT STUDIES IN MONTANA WITH SPECIAL REFERENCE TO WINTER KILLING. (Abstract) Jour. Amer. Soc. Agron. 17: 630-631.
- (9) JONES, M. G., and TINCKER, M. A. H.
1926. YIELD STUDIES IN OATS: THE EFFECT OF PRE-TREATMENT OF THE PARENT CROP UPON THE SEED PRODUCED, ITS GERMINATION AND SUBSEQUENT GROWTH. Ann. Appl. Biol. 13: 535-559, illus.
- (10) KIDD, F., and WEST, C.
1918-19. PHYSIOLOGICAL PRE-DETERMINATION: THE INFLUENCE OF THE PHYSIOLOGICAL CONDITION OF THE SEED UPON THE COURSE OF SUBSEQUENT GROWTH AND UPON THE YIELD. IV. REVIEW OF LITERATURE. Ann. Appl. Biol. 5: [112]-142, [157]-170, 220-251, illus.
- (11) KIESSLING, L.
1911. UNTERSUCHUNG ÜBER DIE KEIMREIFUNG DER GETRIEDE. Landw. Jahrb. Bayern 1: [449]-514.
- (12) KLAGES, K. H.
1926. METRICAL ATTRIBUTES AND THE PHYSIOLOGY OF HARDY VARIETIES OF WINTER WHEAT. Jour. Amer. Soc. Agron. 18: 529-566, illus.
- (13) MUNERATI, O.
1921. THE INFLUENCE OF LOW TEMPERATURES ON THE GERMINATION OF WHEAT AS SOON AS HARVESTED AND ON FRESH SEED IN GENERAL. Internatl. Rev. Sci. and Pract. Agr. [Rome] 12: 161-162.
- (14) POPOFF, M.
1924. STIMULATION OF THE VITAL FUNCTIONS OF CELLS AND ITS IMPORTANCE IN AGRICULTURE. Internatl. Rev. Sci. and Pract. Agr. [Rome] (n. s.) 2: 66-68.

-
- (15) SILBERT, D.
1924. NOTES ON THE STIMULATING INFLUENCE OF SEED TREATMENT UPON THE SUBSEQUENT GROWTH OF PLANTS. N. J. Agr. Expt. Sta. Ann. Rpt. (1923) 44: 259-262.
- (16) TINCKER, M. A. H.
1925. PHYSIOLOGICAL PRE-DETERMINATION EXPERIMENTS WITH CERTAIN ECONOMIC CROPS. THE RELATION BETWEEN RATE OF GERMINATION AND SUBSEQUENT GROWTH. Ann. Appl. Biol. 12: 440-471, illus.
- (17) UNITED STATES DEPARTMENT OF AGRICULTURE.
1927. AGRICULTURAL STATISTICS. U. S. Dept. Agr. Yearbook 1926: 803.
- (18) WANSER, H. M.
1922. PHOTOPERIODISM OF WHEAT; A DETERMINING FACTOR IN ACCLIMATIZATION. Science (n. s.) 56: 313-315.

FURTHER STUDIES OF THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION ¹

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INTRODUCTION

The objects of the research herein reported were to confirm or disprove the results of an earlier investigation (4)³ and to extend the study to include higher planes of nutrition than were reached in the first series of experiments. This second year's work seemed especially desirable for the reason that the results obtained with the two animals used as subjects in the first year's experiments agreed so very closely as to raise a question as to the extent to which the data were fundamentally significant or were determined by the method of experimentation and rigidity of control.

PLAN OF EXPERIMENTATION

The outline of experiments, as set forth in Table 1, provided for a duplicate series of balances of matter and energy, at seven planes of nutrition between fast and three times the energy maintenance requirement, with two steers as subjects. Only one of the two animals, however, would eat as much as three times maintenance.

The general method of experimentation was the same as in the earlier study, except that the sequence of the experimental treatments was changed.

TABLE 1.—*Schedule of experimentation, daily rations, and live weights of animals*

Period No.	Steer No.	Preliminary feeding period on experimental rations	Total digestion period	Calorimeter period	Plane of nutrition	Rations fed daily		Average live weight of animals
						Roughage (alfalfa hay)	Concentrate (corn meal)	
1	60	Sept. 23-Oct. 2.	Oct. 3-21-----	Oct. 18-21-----	Maintenance-----	<i>Kgm.</i> 1.574	<i>Kgm.</i> 1.626	<i>Kgm.</i> 310.9
2	57	Oct. 7-16-----	Oct. 17-Nov. 4--	Nov. 1-4-----	do-----	1.716	1.774	389.6
3	60	Oct. 21-30-----	Oct. 31-Nov. 18.	Nov. 15-18-----	One and a half times maintenance.	2.362	2.440	332.9
4	57	Nov. 5-13-----	Nov. 14-Dec. 2..	Nov. 29-Dec. 2..	do-----	2.574	2.662	384.3
5	60	Nov. 18-27-----	Nov. 28-Dec. 16.	Dec. 13-16-----	Two times maintenance.	3.190	3.290	358.4
6	57	Dec. 3-11-----	Dec. 12-31-----	Dec. 28-31-----	do-----	3.480	3.584	403.1
7	60	Dec. 21-25-----	Dec. 26-Jan. 13..	Jan. 10-13-----	Two and a half times maintenance.	4.180	4.310	391.8
8	57	Jan. 3-8-----	Jan. 9-27-----	Jan. 24-27-----	do-----	4.496	4.636	443.7
9	60	Jan. 18-22-----	Jan. 23-Feb. 10..	Feb. 7-10-----	Three times maintenance.	5.300	5.470	426.6
10	57	Feb. 4-8-----	Feb. 9-Mar. 2..	Feb. 28-Mar. 2..	One-half maintenance	.958	.988	398.3
11	60	Feb. 16-22-----	Feb. 23-Mar. 16.	Mar. 13-16-----	do-----	.948	.978	381.0
12	57	Mar. 4-11-----	Mar. 12-30-----	Mar. 27-30-----	Maintenance (alfalfa hay alone).	5.724	-----	425.6
13	60	Mar. 16-25-----	Mar. 26-Apr. 13.	Apr. 10-13-----	do-----	5.578	-----	412.1
14	57	-----	-----	Apr. 17-21-----	Fast-----	-----	-----	-----
15	60	-----	-----	May 1-5-----	do-----	-----	-----	-----

¹ Received for publication June 7, 1928; issued January, 1930.

² The authors acknowledge their grateful indebtedness to the Department of Dairy Husbandry of this college for cooperation in the conduct of this series of experiments through the contribution of the efficient services of Paul S. Williams.

³ Reference is made by number (italic) to the "Literature cited," p. 78.

During the first 11 periods the rations were composed of approximately equal parts by weight (dry-matter basis) of corn meal and alfalfa hay. In periods 1 and 2 the animals were fed at a plane of maintenance, or energy equilibrium, the succeeding periods following in regular order of increasing rates of feeding, to period 9, in which approximately three times the maintenance requirement of feed was given. The rate of feeding was then decreased, in periods 10 and 11, to half of the maintenance requirement; and in the last of the feeding periods, 12 and 13, a maintenance ration of alfalfa hay alone was fed.

In periods 14 and 15 no feed was given, these being the final periods of fast used to establish the base values from which to reckon the effects of feeding at the several planes of nutrition.

The periods on alfalfa hay alone (12 and 13) were included to provide for the determination of the net-energy value of the hay by itself, and to make possible, in connection with periods 1 and 2, during which the mixed ration was fed, the computation, by difference, of the net-energy value of the corn alone.

In accordance with the standard procedure of this institute the periods of fast followed periods of maintenance, thus providing for a uniform influence of previous feeding on the heat production of fast.

The alfalfa hay used was of the same lot as in the earlier study, but the corn was of different lots which were purchased from time to time during the progress of the experiments. The hay was fed cut, as usual, for convenience in sampling and feeding; and the corn was finely ground.

Each experimental feeding period comprised, as usual, a 10-day preliminary feeding on the experimental ration and an 18-day metabolism period during which urine and feces were collected. The last three days constituted the period of respiration calorimetric measurements.

The heat production of fast was measured during the fourth, fifth, sixth, and seventh days after the withdrawal of feed, but the heat production of the fourth day was used as the base value, to represent the energy metabolism of fast, in the computation of results. This datum will be discussed later.

The details of experimental technic were, with slight exceptions to be noted, the standard procedures of this institute, including the latest revisions of method as enumerated in the earlier paper (4, p. 257).

As in the previous year's work, the value used for the heat production in each of the feeding periods was the average of the direct heat measurement and the heat production as computed by the balance method, but, differing from the practice of the year before, the value used for the heat production of fast was computed from the gravimetrically determined carbon dioxide by the use of the assumed respiratory quotient of 0.707. The necessity and the justification for the assumption of this respiratory quotient will be discussed later.

EXPERIMENTAL SUBJECTS

The steers used, which were designated Nos. 57 and 60, were purchased at the Pittsburgh, Pa., market. They were roan Shorthorns, approximately 2 years of age, and of unknown history. Their average live weights in periods 1 and 2 were 310.9 and 359.6 kgm., respectively.

Steer No. 57 reached an average weight of 443.7 kgm. in period 8, and steer No. 60, an average weight of 426.6 kgm. in period 9, at planes of nutrition of two and a half and three times maintenance, respectively.

The extent to which these increases in live weight during rise in the plane of nutrition, in the course of these experiments, involved increase in the weight and in the dry substance of the contents of the alimentary tract, is not definitely known, but is important in relation to the computation of the energy expense of food utilization—the heat increment—as will be explained later. As a result of recent informal observations, it is the belief of the writers that the animal tends to maintain the gross weight of the contents of the alimentary tract constant, by means of water, in spite of extensive changes in the plane of nutrition, the concentration of the total dry matter in the contents of the alimentary tract decreasing prominently with the lowering of the plane of nutrition.

In computing the heat increment, the difference in feed between two periods is related to the difference in heat production between the same periods. However, since the live weight will naturally differ in the two experimental periods at different planes of nutrition, it is necessary to compute the heat production in both periods to the basis of the same live weight, so that the heat increment, which is defined as the total increase in heat production incident to the utilization of food, will not be affected by a difference in the energy expense of maintenance.

No accurate method is known for taking into account, in this relation, the difference in the live weight, or the difference in the quantity and the composition of the contents of the alimentary tract of the animals, in the experimental periods at the different planes of nutrition which are compared. It is therefore desirable that the live weights of the animals, in the periods compared, should be as nearly the same as practicable, so that the correction of the heat production for difference in live weights may be as small as possible, since this correction involves elements of error.

It was found in the course of the organization of the results that the sequence of experimental periods was unfortunate in two respects, (1) that the maintenance periods on hay alone (12 and 13) were so widely separated from the maintenance periods on hay and corn (1 and 2), since the determination of the net-energy value of the corn depended on the comparison of the heat production of these periods; and (2) since in computing the heat increments between fast and the several levels of feeding, the smallest differences in feed were associated with the greatest differences in the live weight of the animals, thus leading to the maximum possible exaggeration of any such error as exists in the correction of the heat production, as observed, to the basis of a uniform live weight.

This element of error is believed not to be extensive, and is not observable in the curve of heat production, but seems prominently to affect some of the derived values, especially the net-energy values of the mixed ration and of the grain, for maintenance, and of the individual feeds, for body increase, as computed by the heat-increment-proportional method used in the former study. From this point of view the sequence of experimental treatments in the previous study was much more satisfactory.

It appears, however, that the gain from this unfortunate situation is immeasurably greater than the loss, since a new and important requirement in the planning of experiments for the determination of net-energy values of feeds has been brought clearly to light. This matter will be discussed further in relation to the problem of determining net-energy values.

TABLE 2.—*Digestibility of rations*

Period No.	Steer No.	Item	Dry matter	Organic matter	Crude protein	Crude fiber	Ether extract	N-free extract	Carbon	Energy	Nitrogen
1	60	Salt.....grams.....	30								
		Alfalfa hay.....do.....	1,420	1,290.2	208.4	493.1	20.0	568.8	652.0	6,294.0	33.3
		Corn meal.....do.....	1,408	1,385.8	139.5	31.4	57.5	1,157.3	649.2	6,335.6	22.3
		Total fed.....do.....	2,858	2,676.0	347.9	524.5	77.5	1,726.1	1,301.2	12,629.6	55.6
		Feces.....do.....	694	627.7	111.5	291.0	27.4	197.8	344.9	3,461.4	17.8
		Digestibility (per cent)	75.7	76.5	68.0	44.5	64.6	88.5	73.5	72.6	68.0
	57	Salt.....grams.....	30								
		Alfalfa hay.....do.....	1,546	1,404.7	226.9	536.8	21.7	619.3	709.8	6,852.5	36.3
		Corn meal.....do.....	1,539	1,514.7	152.5	34.3	62.9	1,265.0	709.6	6,925.0	24.4
		Total fed.....do.....	3,115	2,919.4	379.4	571.1	84.6	1,884.3	1,419.4	13,777.5	60.7
		Feces.....do.....	750	676.1	111.6	310.5	31.2	222.8	362.8	3,639.2	17.9
2	60	Salt.....grams.....	30								
		Alfalfa hay.....do.....	2,134	1,938.9	313.2	741.0	30.0	854.8	979.8	9,458.7	50.1
		Corn meal.....do.....	2,103	2,070.1	207.8	46.4	87.2	1,728.6	974.0	9,473.8	33.2
		Total fed.....do.....	4,267	4,009.0	521.0	787.4	117.2	2,583.4	1,953.8	18,932.5	83.3
		Feces.....do.....	1,132	1,030.9	187.5	409.9	45.5	387.7	550.1	5,465.9	30.0
		Digestibility (per cent)	73.5	74.3	64.0	47.9	61.2	85.0	71.8	71.1	64.0
	57	Salt.....grams.....	30								
		Alfalfa hay.....do.....	2,319	2,107.0	340.4	805.2	32.6	928.9	1,064.8	10,278.7	54.5
		Corn meal.....do.....	2,293	2,257.1	226.6	50.6	95.1	1,884.8	1,062.0	10,329.7	36.3
		Total fed.....do.....	4,642	4,364.1	566.9	855.8	127.7	2,813.7	2,126.8	20,608.4	90.8
		Feces.....do.....	1,118	1,012.1	190.0	418.3	52.0	351.8	549.5	5,483.6	36.4
3	60	Salt.....grams.....	30								
		Alfalfa hay.....do.....	2,866	2,604.0	420.6	995.1	40.3	1,148.0	1,315.9	12,703.3	67.3
		Corn meal.....do.....	2,838	2,793.6	280.4	62.7	72.2	2,532.8	1,314.5	12,784.9	44.9
		Total fed.....do.....	5,734	5,397.6	701.0	1,057.8	115.0	3,480.8	2,630.4	25,488.2	112.2
		Feces.....do.....	1,551	1,413.7	261.2	564.3	57.8	580.5	751.9	7,484.4	41.8
		Digestibility (per cent)	73.0	73.8	62.7	46.7	63.4	84.8	71.4	70.0	62.7
	57	Salt.....grams.....	30								
		Alfalfa hay.....do.....	3,144	2,856.6	461.4	1,061.7	44.2	1,259.3	1,448.6	13,935.5	73.8
		Corn meal.....do.....	3,089	3,040.6	305.2	68.2	128.1	2,539.1	1,430.7	13,915.0	48.8
		Total fed.....do.....	6,263	5,897.2	766.6	1,159.9	172.3	3,798.4	2,879.3	27,850.5	122.6
		Feces.....do.....	1,637	1,484.2	273.2	589.6	79.9	541.5	808.8	8,029.3	43.7
4	60	Salt.....grams.....	30								
		Alfalfa hay.....do.....	3,793	3,446.2	556.6	1,317.0	53.3	1,519.3	1,741.6	16,812.1	89.1
		Corn meal.....do.....	3,727	3,668.6	368.3	82.3	154.6	3,063.5	1,726.2	16,789.8	58.9
		Total fed.....do.....	7,520	7,114.8	924.9	1,399.3	207.9	4,582.8	3,467.8	33,601.9	148.0
		Feces.....do.....	2,117	1,922.9	365.3	752.8	78.1	726.7	1,040.8	10,337.0	58.5
		Digestibility (per cent)	72.0	73.0	60.5	46.2	62.4	84.1	70.0	69.2	60.5
	57	Salt.....grams.....	30								
		Alfalfa hay.....do.....	4,051	3,680.7	594.5	1,406.6	57.0	1,622.6	1,860.0	17,955.7	95.1
		Corn meal.....do.....	4,006	3,944.3	397.6	91.5	170.3	3,284.9	1,855.9	18,069.9	63.6
		Total fed.....do.....	8,087	7,625.0	992.1	1,498.1	227.3	4,907.5	3,715.9	36,025.6	158.7
		Feces.....do.....	2,248	2,049.5	377.0	825.4	84.1	763.1	1,096.8	10,879.8	60.3
5	60	Salt.....grams.....	30								
		Alfalfa hay.....do.....	4,763	4,327.6	699.0	1,653.8	67.0	1,907.8	2,186.9	21,111.5	111.8
		Corn meal.....do.....	4,726	4,653.2	469.1	108.0	200.9	3,875.3	2,189.5	21,317.6	75.0
		Total fed.....do.....	9,519	8,980.8	1,168.1	1,761.8	267.9	5,783.1	4,376.4	42,429.1	186.8
		Feces.....do.....	2,926	2,654.3	476.8	1,026.0	97.4	1,054.1	1,416.3	13,958.5	76.3
		Digestibility (per cent)	69.3	70.4	59.2	41.8	63.6	81.8	67.6	67.1	59.2
	57	Salt.....grams.....	30								
		Alfalfa hay.....do.....	871	791.4	127.8	302.4	12.2	348.9	399.9	3,860.6	20.5
		Corn meal.....do.....	829	815.4	80.3	20.2	34.6	680.4	383.0	3,734.9	12.8
		Total fed.....do.....	1,730	1,606.8	208.1	322.6	46.8	1,029.3	782.9	7,595.5	33.3
		Feces.....do.....	424	379.4	65.5	185.7	14.8	113.4	207.7	2,072.0	10.5
6	60	Salt.....grams.....	30								
		Alfalfa hay.....do.....	862	783.2	126.5	299.3	12.1	345.3	395.8	3,820.7	20.2
		Corn meal.....do.....	819	805.6	79.3	19.9	34.2	672.2	378.4	3,689.8	12.7
		Total fed.....do.....	1,711	1,588.8	205.8	319.2	46.3	1,017.5	774.2	7,510.5	32.9
		Feces.....do.....	436	395.6	72.6	184.1	18.2	120.6	213.4	2,128.2	11.6
		Digestibility (per cent)	74.5	75.1	64.7	42.3	60.7	88.1	72.4	71.7	64.7
	57	Salt.....grams.....	30								
		Alfalfa hay.....do.....	5,155	4,683.7	756.5	1,789.8	72.5	2,064.8	2,366.9	22,849.0	121.0
		Corn meal.....do.....	2,050	1,860.8	257.9	956.4	72.8	573.8	1,005.3	9,905.0	41.3
		Digestibility (per cent)	60.5	60.3	65.9	46.6		72.2	57.5	56.7	65.9
		Salt.....grams.....	30								
7	60	Salt.....grams.....	30								
		Alfalfa hay.....do.....	5,013	4,554.7	735.7	1,740.6	70.5	2,008.0	2,301.7	22,219.6	117.7
		Corn meal.....do.....	2,077	1,879.3	263.9	943.3	82.5	589.6	1,013.3	10,002.5	42.2
		Digestibility (per cent)	58.0	58.8	64.1	45.8		70.6	56.0	55.0	64.1
		Salt.....grams.....	30								
	57	Salt.....grams.....	30								
		Alfalfa hay.....do.....	5,155	4,683.7	756.5	1,789.8	72.5	2,064.8	2,366.9	22,849.0	121.0
		Corn meal.....do.....	2,050	1,860.8	257.9	956.4	72.8	573.8	1,005.3	9,905.0	41.3
		Digestibility (per cent)	60.5	60.3	65.9	46.6		72.2	57.5	56.7	65.9
		Salt.....grams.....	30								

TABLE 3.—Carbon dioxide, water vapor, and methane eliminated per day

Steer No.	Period No.	Calorimeter day	CO ₂	C as CO ₂	H ₂ O	CH ₄	C as CH ₄
			<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
60	1	First.....	2,945.9	803.3	2,759.3	79.5	56.8
		Second.....	2,922.7	797.0	2,718.4	83.1	62.2
		Third.....	2,934.3	800.2	2,738.9	79.5	59.8
		Average.....	2,934.3	800.2	2,738.9	79.5	59.8
57	2	First.....	3,271.5	892.1	3,761.7	96.0	71.3
		Second.....	3,271.9	892.2	3,536.9	94.1	71.3
		Third.....	3,196.7	871.8	3,435.0	95.8	71.3
		Average.....	3,246.7	885.4	3,577.9	95.3	71.3
60	3	First.....	3,749.9	1,022.6	4,100.6	110.1	82.4
		Second.....	3,761.6	1,025.8	4,039.0	110.1	82.4
		Third.....	3,740.0	1,019.9	4,294.6	112.1	83.9
		Average.....	3,750.5	1,022.8	4,144.7	111.4	83.2
57	4	First.....	4,122.4	1,124.2	5,471.0	136.5	102.2
		Second.....	4,126.5	1,125.3	5,193.4	132.7	99.3
		Third.....	4,064.8	1,108.4	5,044.2	131.4	98.3
		Average.....	4,104.5	1,119.3	5,236.2	133.5	99.9
60	5	First.....	4,847.9	1,322.0	7,659.0	156.8	117.3
		Second.....	4,837.3	1,319.1	7,086.5	155.8	116.6
		Third.....	4,897.2	1,335.5	7,059.2	156.4	117.1
		Average.....	4,880.8	1,325.5	7,268.2	156.3	117.0
57	6	First.....	5,223.4	1,424.4	8,809.9	170.4	127.5
		Second.....	5,396.7	1,471.7	8,746.7	173.7	130.0
		Third.....	5,310.1	1,448.1	8,987.1	181.0	135.4
		Average.....	5,310.1	1,448.1	8,847.9	175.0	131.0
60	7	First.....	6,279.7	1,712.5	11,596.7	186.8	139.8
		Second.....	6,180.9	1,671.9	10,872.0	185.2	138.6
		Third.....	6,142.7	1,675.1	10,493.9	194.2	145.4
		Average.....	6,184.4	1,686.5	10,987.5	188.7	141.3
57	8	First.....	6,267.1	1,709.0	10,459.9	212.4	159.0
		Second.....	6,678.6	1,821.3	11,090.5	226.8	169.8
		Third.....	6,472.9	1,765.2	10,775.2	219.6	164.4
		Average.....	6,472.9	1,765.2	10,775.2	219.6	164.4
60	9	First.....	7,276.1	1,984.2	8,151.3	232.7	174.1
		Second.....	7,290.0	1,988.0	8,775.6	228.3	170.9
		Third.....	7,326.3	1,997.9	8,810.4	230.2	172.3
		Average.....	7,297.5	1,990.0	8,579.1	230.4	172.4
57	10	First.....	2,891.1	788.4	3,831.4	57.4	43.0
		Second.....	2,845.3	775.9	3,762.3	55.9	41.8
		Third.....	2,848.9	776.9	3,655.9	54.1	40.5
		Average.....	2,861.8	780.4	3,749.9	55.8	41.8
60	11	First.....	2,771.5	755.8	2,894.1	56.4	42.2
		Second.....	2,717.2	741.0	2,850.3	55.4	41.4
		Third.....	2,667.7	727.5	2,815.5	54.0	40.4
		Average.....	2,718.8	741.4	2,853.3	55.3	41.3
57	12	First.....	4,066.8	1,109.0	4,930.0	121.3	90.8
		Second.....	4,009.4	1,093.4	4,805.0	118.1	88.4
		Third.....	3,979.9	1,085.3	4,867.2	118.7	88.8
		Average.....	4,018.7	1,095.9	4,867.4	119.4	89.3
60	13	First.....	3,860.8	1,052.8	4,667.7	109.5	81.9
		Second.....	3,899.0	1,063.2	4,763.5	109.9	82.2
		Third.....	3,892.5	1,061.5	4,949.4	110.0	82.3
		Average.....	3,884.1	1,059.2	4,793.5	109.8	82.1

TABLE 4.—*Energy of the urine and of the protein corrected for the incomplete oxidation of protein gained or lost*

Period No.	Balance of N	Correction (N × 7.45)	Energy of urine		Energy of protein	
			Uncorrected for N equilibrium	Corrected	Uncorrected grams protein (× 5.7)	Corrected
	Grams	Calories	Calories	Calories	Calories	Calories
.....	-2.6	-19.4	581.7	562.3	88.9	60.5
.....	-4.9	-36.5	659.9	623.4	167.6	131.1
2.....	+13.5	+100.6	652.8	753.4	461.7	361.1
3.....	+15.5	+115.5	758.5	874.0	530.1	414.6
4.....	+20.7	+154.2	862.3	1,016.5	707.9	553.7
5.....	+13.6	+100.6	984.8	1,123.4	636.1	497.5
6.....	+23.0	+171.4	1,108.4	1,279.8	786.6	615.2
7.....	+19.1	+142.3	1,217.8	1,360.1	653.2	510.9
8.....	+22.0	+164.6	1,335.6	1,500.2	755.8	591.2
9.....	-16.9	-125.9	512.5	386.6	578.0	452.1
10.....	-17.8	-132.6	506.6	374.0	608.8	476.2
11.....	+1.0	+7.5	1,155.1	1,162.6	34.2	26.7
12.....	+2.9	+21.6	1,072.6	1,094.2	99.2	77.6
13.....						

TABLE 5.—*Balance of matter and energy per day*

PERIOD 1, STEER NO. 60

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	Grams	Grams	Grams	Calories
Income:					
Alfalfa hay.....	1,420	161	33.3	652.0	6,294.0
Corn meal.....	1,408	216	22.3	649.2	6,335.6
Water.....		5,743			
Total.....	2,828	6,120	55.6	1,301.2	12,629.6
Outgo:					
Feces.....	694	1,795	17.8	344.9	3,461.4
Urine.....		2,691	40.4	66.8	562.3
Methane.....	79.5			59.5	1,060.8
Carbon dioxide.....	2,934.3			800.2	
Water vapor.....		2,739			
Metabolizable: Income minus urine, feces, and methane.....					7,545.1
Body balances:					
Fat.....	+49.7			+38.0	+472.2
Protein.....	-15.6		-2.6	-8.2	-100.5
Water.....		-1,105			
Computed heat production.....					7,142.4
Observed heat production.....					7,252.9

PERIOD 2, STEER NO. 57

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	Grams	Grams	Grams	Calories
Income:					
Alfalfa hay.....	1,546	195	36.3	709.8	6,852.5
Corn meal.....	1,539	245	24.4	709.6	6,925.0
Water.....		6,850			
Total.....	3,085	7,290	60.7	1,419.4	13,777.5
Outgo:					
Feces.....	750	2,968	17.9	362.8	3,639.2
Urine.....		3,780	47.7	72.6	623.4
Methane.....	95.3			71.3	1,271.7
Carbon dioxide.....	3,246.7			885.4	
Water vapor.....		3,578			
Metabolizable: Income minus urine, feces, and methane.....					8,243.2
Body balances:					
Fat.....	+55.8			+42.7	+530.1
Protein.....	-29.4		-4.9	-15.4	-131.1
Water.....		-3,036			
Computed heat production.....					7,844.2
Observed heat production.....					7,908.7

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TABLE 5.—Balance of matter and energy per day—Continued

PERIOD 3, STEER NO. 60

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	Grams	Grams	Grams	Grams	Calories
Alfalfa hay.....	2,154	233	50.1	979.8	9,458.7
Corn meal.....	2,103	340	33.2	974.0	9,473.8
Water.....		13,237			
Total.....	4,237	13,810	83.3	1,953.8	18,932.5
Outgo:					
Feces.....	1,132	5,821	30.0	550.1	5,465.9
Urine.....		5,118	39.8	74.7	742.9
Methane.....	111.1			83.2	1,482.5
Carbon dioxide.....	3,750.5			1,022.8	
Water vapor.....		4,145			
Metabolizable: Income minus urine, feces, and methane.....					11,241.2
Body balances:					
Fat.....	+235.8			+180.4	+2,240.1
Protein.....	+81.0		+13.5	+42.6	+361.1
Water.....		-1,274			
Computed heat production.....					8,640.0
Observed heat production.....					8,821.4

PERIOD 4, STEER NO. 57

Income:					
Alfalfa hay.....	2,319	267	54.5	1,064.8	10,278.7
Corn meal.....	2,293	381	36.3	1,062.0	10,329.7
Water.....		8,143			
Total.....	4,612	8,791	90.8	2,126.8	20,608.4
Outgo:					
Feces.....	1,118	4,276	30.4	549.5	5,483.6
Urine.....		5,152	44.9	86.8	874.0
Methane.....	133.5			99.9	1,781.4
Carbon dioxide.....	4,104.5			1,119.3	
Water vapor.....		5,236			
Metabolizable: Income minus urine, feces, and methane.....					12,469.4
Body balances:					
Fat.....	+290.7			+222.4	+2,761.7
Protein.....	+93.0		+15.5	+48.9	+414.6
Water.....		-5,873			
Computed heat production.....					9,293.1
Observed heat production.....					9,493.3

PERIOD 5, STEER NO. 60

Income:					
Alfalfa hay.....	2,866	349	67.3	1,315.9	12,703.3
Corn meal.....	2,838	465	44.9	1,314.5	12,784.9
Water.....		15,623			
Total.....	5,704	16,437	112.2	2,630.4	25,488.2
Outgo:					
Feces.....	1,551	5,683	41.8	751.9	7,484.4
Urine.....		4,840	49.7	99.2	1,016.5
Methane.....	156.3			117.0	2,085.7
Carbon dioxide.....	4,860.8			1,325.5	
Water vapor.....		7,268			
Metabolizable: Income minus urine, feces, and methane.....					14,901.6
Body balances:					
Fat.....	+354.9			+271.5	+3,371.6
Protein.....	+124.2		+20.7	+65.3	+553.7
Water.....		-1,354			
Computed heat production.....					10,976.3
Observed heat production.....					11,156.9

TABLE 5.—Balance of matter and energy per day—Continued

PERIOD 6, STEER NO. 57

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>
Income:					
Alfalfa hay	3,144	342	73.8	1,443.6	13,935.5
Corn meal	3,089	595	48.8	1,430.7	13,915.6
Water		17,490			
Total	6,233	18,337	122.6	2,874.3	27,851.1
Outgo:					
Feces	1,637	5,630	43.7	803.8	8,029.3
Urine		6,234	60.3	111.7	1,123.4
Methane	175.0			131.0	2,335.2
Carbon dioxide	5,310.1			1,448.1	
Water vapor		8,848			
Metabolizable: Income minus urine, feces, and methane					16,363.2
Body balances:					
Fat	+419.7			+321.1	+3,987.2
Protein	+111.6		+18.6	+58.6	+497.5
Water		-2,375			
Computed heat production					11,878.5
Observed heat production					11,851.2

PERIOD 7, STEER NO. 60

Income:					
Alfalfa hay	3,793	393	89.1	1,741.6	16,812.1
Corn meal	3,727	596	58.9	1,726.2	16,789.8
Water		20,813			
Total	7,520	21,802	148.0	3,467.8	33,601.9
Outgo:					
Feces	2,117	7,685	58.5	1,040.8	10,337.0
Urine		5,858	66.5	126.6	1,279.8
Methane	188.7			141.3	2,518.0
Carbon dioxide	6,184.4			1,686.5	
Water vapor		10,988			
Metabolizable: Income minus urine, feces, and methane					19,467.1
Body balances:					
Fat	+522.9			+400.1	+4,967.6
Protein	+138.0		+23.0	+72.5	+615.2
Water		-2,729			
Computed heat production					13,884.3
Observed heat production					13,970.4

PERIOD 8, STEER NO. 57

Income:					
Alfalfa hay	4,051	• 771	95.1	1,860.0	17,955.7
Corn meal	4,006	• 943	63.6	1,855.9	18,069.9
Water		25,195			
Total	8,057	26,914	158.7	3,715.9	36,025.6
Outgo:					
Feces	2,243	7,826	60.3	1,096.3	10,879.8
Urine		6,774	79.3	140.8	1,360.1
Methane	219.6			164.4	2,930.3
Carbon dioxide	6,472.9			1,765.2	
Water vapor		10,775			
Metabolizable: Income minus urine, feces, and methane					20,855.4
Body balances:					
Fat	+639.1			+489.0	+6,071.5
Protein	+114.6		+19.1	+60.2	+510.9
Water		+1,439			
Computed heat production					14,273.0
Observed heat production					14,408.2

• Includes 313 grams of water added to refused feed fed back.

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*Energy Metabolism of Cattle*TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 9, STEER NO. 60

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>
Income:					
Alfalfa hay	4,763	537	111.8	2,186.9	21,111.5
Corn meal	4,726	744	75.0	2,189.5	21,317.6
Water		22,817			
Total	9,489	24,098	186.8	4,376.4	42,429.1
Outgo:					
Feces	2,926		76.3	1,416.3	13,958.5
Urine			88.4	150.4	1,500.2
Methane	230.4			172.4	3,074.5
Carbon dioxide	7,297.5			1,990.0	
Water vapor		8,579			
Metabolizable: Income minus urine, feces, and methane					23,895.9
Body balances:					
Fat	+754.9			+577.6	+7,171.6
Protein	+132.6		+22.1	+69.7	+591.2
Water					
Computed heat production					16,133.1
Observed heat production					

PERIOD 10, STEER NO. 57

Income:					
Alfalfa hay	871	87	20.5	399.9	3,860.6
Corn meal	829	151	12.8	383.0	3,734.9
Water		9,300			
Total	1,700	9,538	33.3	782.9	7,595.5
Outgo:					
Feces	424	1,623	10.5	207.7	2,072.0
Urine		3,421	39.7	55.8	386.6
Methane	55.8			41.8	744.6
Carbon dioxide	2,861.8			780.4	
Water vapor		3,750			
Metabolizable: Income minus urine, feces, and methane					4,392.3
Body balances:					
Fat	-326.1			-249.5	-3,098.0
Protein	-101.4		-16.9	-53.3	-452.1
Water		+744			
Computed heat production					7,942.4
Observed heat production					7,939.1

PERIOD 11, STEER NO. 60

Income:					
Alfalfa hay	862	90	20.2	395.8	3,820.7
Corn meal	819	166	12.7	378.4	3,689.8
Water		9,697			
Total	1,681	9,953	32.9	774.2	7,510.5
Outgo:					
Feces	436	1,905	11.6	213.4	2,128.2
Urine		3,780	39.1	53.3	374.0
Methane	55.3			41.3	737.9
Carbon dioxide	2,718.8			741.4	
Water vapor		2,853			
Metabolizable: Income minus urine, feces, and methane					4,270.4
Body balances:					
Fat	-286.4			-219.1	-2,720.8
Protein	-106.8		-17.8	-56.1	-476.2
Water		+1,415			
Computed heat production					7,467.4
Observed heat production					7,476.2

TABLE 5.—Balance of matter and energy per day—Continued

PERIOD 12, STEER NO. 57

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>
Alfalfa hay.....	5,155	577	121.0	2,366.9	22,849.0
Water.....		18,067			
Total.....	5,155	18,644	121.0	2,366.9	22,849.0
Outgo:					
Feces.....	2,050	8,417	41.3	1,005.3	9,905.0
Urine.....		7,350	78.7	135.0	1,162.6
Methane.....	119.4			89.3	1,593.3
Carbon dioxide.....	4,018.7			1,095.9	
Water vapor.....		4,867			
Metabolizable: Income minus, urine, feces, and methane.....					10,188.1
Body balances:					
Fat.....	+49.9			+38.2	+474.1
Protein.....	+6.0		+1.0	+3.2	+26.7
Water.....		-1,990			
Computed heat production.....					9,687.3
Observed heat production.....					9,953.7

PERIOD 13, STEER NO. 60

Income:					
Alfalfa hay.....	5,013	569	117.7	2,301.7	22,219.6
Water.....		17,155			
Total.....	5,013	17,724	117.7	2,301.7	22,219.6
Outgo:					
Feces.....	2,077	8,702	42.2	1,013.3	10,002.5
Urine.....		7,156	72.6	130.8	1,094.2
Methane.....	109.8			82.1	1,465.2
Carbon dioxide.....	3,884.1			1,059.2	
Water vapor.....		4,794			
Metabolizable: Income minus urine, feces, and methane.....					9,657.7
Body balances:					
Fat.....	+9.4			+7.2	+89.3
Protein.....	+17.4		+2.9	+9.1	+77.6
Water.....		-2,928			
Computed heat production.....					9,490.8
Observed heat production.....					9,790.1

TABLE 6.—Heat emission and heat production per day

Steer No.	Period No.	Calorimeter days	Sub-period No.	Heat emission			Corrections		Heat production
				By radiation and conduction	As latent heat of water vapor	Total	Body gain	Platform	
				<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>
60	1	First	1	2,845.2					
			2	2,840.7					
			5	5,685.9	1,619.8	7,305.7			
			6	2,820.0					
		Third	6	2,829.7					
			6	5,649.7	1,595.6	7,245.3			
		Daily average		5,667.8	1,607.7	7,275.5	-22.6	0	7,252.9
57	2	First	1	3,012.2					
			2	2,976.4					
			3	5,988.6	2,208.0	8,196.6			
			4	2,886.9					
		Second	4	2,960.0					
			5	5,846.9	2,076.2	7,923.1			
		Third	6	2,874.2					
			6	2,873.4					
			6	5,747.7	2,006.2	7,753.9			
		Daily average		5,861.1	2,096.8	7,957.9	-63.7	+14.5	7,908.7
60	3	First	1	3,028.8					
			2	3,273.3					
			3	6,302.1	2,407.0	8,709.1			
			4	3,127.8					
		Second	4	3,288.1					
			5	6,423.9	2,371.0	8,796.9			
		Third	6	3,166.3					
			6	3,340.8					
			6	6,507.1	2,521.0	9,028.1			
		Daily average		6,411.7	2,433.0	8,844.7	-23.3	0	8,821.4
57	4	First	1	3,045.1					
			2	3,287.2					
			3	6,332.3	3,211.6	9,543.9			
			4	3,165.8					
		Second	4	3,442.9					
			5	6,608.7	3,048.6	9,657.3			
		Third	6	3,327.2					
			6	3,314.5					
			6	6,641.7	2,961.0	9,602.7			
		Daily average		6,527.6	3,073.7	9,601.3	-121.3	+13.3	9,493.3
60	5	First	1	3,495.2					
			2	3,768.6					
			3	7,263.8	3,915.3	11,179.1			
			4	3,425.8					
		Second	4	3,744.0					
			5	7,169.8	3,967.2	11,137.0			
		Third	6	3,401.0					
			6	3,793.0					
			6	7,194.0	4,029.8	11,223.8			
		Daily average		7,209.2	3,970.8	11,180.0	-23.1	0	11,156.9
57	6	First	1	3,797.9					
			2	4,094.6					
			3	7,892.5	3,838.4	11,730.9			
			4	3,810.8					
		Second	4	4,148.4					
			5	7,959.2	3,801.2	11,760.4			
		Third	6	4,012.0					
			6	4,223.4					
			6	8,235.4	3,942.4	12,177.8			
		Daily average		8,029.0	3,860.7	11,889.7	-44.9	+6.4	11,851.2

TABLE 6.—Heat emission and heat production per day—Continued

Steer No.	Period No.	Calorimeter days	Sub-period No.	Heat emission			Corrections		Heat production
				By radiation and conduction	As latent heat of water vapor	Total	Body gain	Platform	
				Calories	Calories	Calories	Calories	Calories	Calories
60	7	First	1	4,504.9					
			2	4,926.3					
			3	9,431.2	4,987.2	14,418.4			
			4	4,306.1					
			5	4,661.2					
		Second	6	8,867.3	4,884.5	13,751.8			
			7	4,335.8					
			8	4,734.3					
		Third	9	9,070.1	4,807.4	13,877.5			
		Daily average		9,122.9	4,893.0	14,015.9	-54.0	+14.5	13,976.4
57	8	First	1	4,606.0					
			2	4,664.0					
			3	9,270.0	4,722.8	13,992.8			
			4	4,549.9					
			5	5,014.1					
		Second	6	9,564.0	4,705.2	14,269.2			
			7	4,827.4					
			8	5,150.4					
		Third	9	9,977.8	4,850.8	14,828.6			
		Daily average		9,603.9	4,759.6	14,363.5	+44.7	0	14,408.2
57	10	First	1	2,868.1					
			2	2,755.5					
			3	5,623.6	2,250.9	7,874.5			
			4	2,825.1					
			5	2,848.5					
		Second	6	5,673.6	2,210.3	7,883.9			
			7	2,941.7					
			8	2,893.9					
		Third	9	5,835.6	2,147.8	7,983.4			
		Daily average		5,710.9	2,203.0	7,913.9	+10.7	+14.5	7,939.1
60	11	First	1	2,849.0					
			2	2,917.3					
			3	5,769.3	1,701.8	7,468.1			
			4	2,974.2					
			5	2,853.3					
		Second	6	5,927.5	1,676.0	7,603.5			
			7	2,734.4					
			8	2,890.3					
		Third	9	5,624.7	1,655.5	7,280.2			
		Daily average		5,772.8	1,677.8	7,450.6	+25.6	0	7,476.2
57	12	First	1	3,327.0					
			2	3,787.1					
			3	7,114.1	2,898.8	10,012.9			
			4	3,325.5					
			5	3,796.6					
		Second	6	7,122.1	2,825.4	9,947.5			
			7	3,548.9					
			8	3,638.7					
		Third	9	7,187.6	2,862.0	10,049.6			
		Daily average		7,141.3	2,862.1	10,003.3	-42.6	-7.0	9,953.7
60	13	First	1	3,505.5					
			2	3,613.3					
			3	7,118.8	2,744.6	9,863.4			
			4	3,375.2					
			5	3,634.3					
		Second	6	7,009.5	2,801.0	9,810.5			
			7	3,389.0					
			8	3,588.9					
		Third	9	6,977.9	2,910.2	9,888.1			
		Daily average		7,035.4	2,818.6	9,854.0	-63.9	0	9,790.1

TABLE 7.—Correction of heat production to a standard day of 12 hours standing and 12 hours lying

Period No.	Steer No.	Time spent standing	Difference from 12 hours	Factor *	Correction	Observed heat production		Computed heat production	
						Uncorrected	Corrected	Uncorrected	Corrected
		Hours	Hours		Calories	Calories	Calories	Calories	Calories
1-----	60	7.9	4.1	20.6	+84.5	7,252.9	7,337.4	7,142.4	7,226.9
2-----	57	8.8	3.2	60.6	+193.9	7,908.7	8,102.6	7,844.2	8,038.1
3-----	60	5.6	6.4	22.1	+141.4	8,821.4	8,962.8	8,640.0	8,781.4
4-----	57	7.4	4.6	64.8	+298.1	9,493.3	9,791.4	9,293.1	9,591.2
5-----	60	5.0	7.0	23.8	+166.6	11,156.9	11,323.5	10,976.3	11,142.9
6-----	57	6.1	5.9	68.0	+401.2	11,851.2	12,252.4	11,878.5	12,279.7
7-----	60	6.5	5.5	26.0	+143.0	13,976.4	14,119.4	13,884.3	14,027.3
8-----	57	8.4	3.6	74.8	+269.3	14,408.2	14,677.5	14,273.0	14,542.3
9-----	60	8.8	3.2	28.3	+90.6			16,133.1	16,223.7
10-----	57	10.4	1.6	67.2	+107.5	7,939.1	8,046.6	7,942.4	8,049.9
11-----	60	8.2	3.8	25.3	+95.1	7,476.2	7,572.3	7,467.4	7,563.5
12-----	57	7.0	5.0	71.8	+359.0	9,953.7	10,312.7	9,687.3	10,046.3
13-----	60	7.4	4.6	27.3	+125.6	9,790.1	9,915.7	9,490.8	9,616.4

* Based on CO₂ production during fast, and computed directly in proportion to the live weight.

TABLE 8.—Urinary nitrogen per 12 hour subperiod, and carbon dioxide, oxygen, and energy equivalents during fast

Steer No. and period	Average urinary nitrogen per 12-hour subperiod	Carbon dioxide equivalent (N×4.75)	Oxygen equivalent (N×5.94)	Energy equivalent (N×26.51)
	Grams	Liters	Liters	Calories
Steer 57, period 14-----	19.6	93.1	116.4	519.6
Steer 60, period 15-----	21.6	102.6	128.3	572.6

TABLE 9.—Carbon dioxide, water vapor, and methane eliminated per day during fast

Steer No. and period	Subperiod and day of fast	Carbon dioxide	Water vapor	Methane
		Grams	Grams	Grams
Steer 57, period 14-----	Subperiod 1-----	1,184.55	1,685.35	1.23
	Subperiod 2-----	1,114.26	1,540.92	.76
	Fourth day-----	2,298.81	3,226.27	1.99
	Subperiod 3-----	1,184.90	1,786.91	.28
	Subperiod 4-----	1,111.62	1,903.18	.61
	Fifth day-----	2,296.52	3,690.09	.89
	Subperiod 5-----	1,207.69	2,677.31	.61
	Subperiod 6-----	1,008.90	1,672.01	1.09
	Sixth day-----	2,211.59	4,249.32	1.70
	Subperiod 7-----	1,090.49	1,484.67	.61
	Subperiod 8-----	1,057.29	1,381.34	.13
	Seventh day-----	2,147.78	2,866.01	.74
	Subperiod 1-----	1,081.07	1,813.10	1.35
	Subperiod 2-----	1,038.26	1,717.03	.55
Steer 60, period 15-----	Fourth day-----	2,119.33	3,530.13	1.90
	Subperiod 3-----	1,047.03	1,462.72	None.
	Subperiod 4-----	1,032.40	1,371.22	None.
	Fifth day-----	2,079.43	2,833.94	None.
	Subperiod 5-----	1,023.04	1,207.83	None.
	Subperiod 6-----	992.00	1,103.85	None.
	Sixth day-----	2,015.04	2,311.68	None.
	Subperiod 7-----	994.95	1,078.52	None.
	Subperiod 8-----	986.47	1,035.61	None.
	Seventh day-----	1,981.42	2,114.13	None.

TABLE 10.—Heat production computed from the CO₂ eliminated, assuming a nonprotein respiratory quotient of 0.707 eliminated

Steer No., period, and subperiod	Nonprotein CO ₂ eliminated	Energy of nonprotein CO ₂ (6.628) ^a	Energy of protein	Heat production
	Liters	Calories	Calories	Calories
Steer 57, period 14:				
Subperiod 1.....	501.7	3,325.3	519.6	3,844.9
Subperiod 2.....	470.3	3,117.1	519.6	3,636.7
Subperiod 3.....	502.6	3,331.2	519.6	3,850.8
Subperiod 4.....	469.5	3,111.8	519.6	3,631.4
Subperiod 5.....	531.4	3,522.1	519.6	4,041.7
Subperiod 6.....	415.1	2,761.3	519.6	3,270.9
Subperiod 7.....	458.5	3,038.9	519.6	3,558.5
Subperiod 8.....	444.5	2,946.1	519.6	3,465.7
Steer 60 period 15:				
Subperiod 1.....	442.4	2,932.2	572.6	3,504.8
Subperiod 2.....	426.5	2,826.8	572.6	3,399.4
Subperiod 3.....	428.0	2,836.8	572.6	3,409.4
Subperiod 4.....	421.4	2,793.0	572.6	3,365.6
Subperiod 5.....	424.3	2,812.3	572.6	3,384.9
Subperiod 6.....	396.9	2,630.7	572.6	3,203.3
Subperiod 7.....	404.4	2,680.4	572.6	3,253.0
Subperiod 8.....	395.2	2,619.4	572.6	3,192.0

^a Calorific value of O₂=4.686 Cals. per liter O₂; calorific value of nonprotein CO₂= $\frac{4.686}{0.707}$ =6.628 Cals. per liter CO₂.

TABLE 11.—Computed heat production^a of fast corrected to the standard day

Steer No., period, and day of fast	Average weight of animal, 4th and 7th days	Time spent standing	Difference from standard of time spent standing	Correction ^b	Heat production, uncorrected	Heat production, corrected to standard day
	Kilograms	Hours	Hours	Calories	Calories	Calories
Steer 57, period 14:						
Fourth day.....	399.2	6.7	5.3	+356.7	7,481.6	7,838.3
Fifth day.....		9.5	2.5	+168.3	7,482.2	7,650.5
Sixth day.....		11.5	.5	+33.7	7,312.6	7,346.3
Seventh day.....		11.5	.5	+33.7	7,024.2	7,057.9
Steer 60, period 15:						
Fourth day.....	383.0	6.9	5.1	+129.5	6,904.2	7,033.7
Fifth day.....		8.6	3.4	+86.4	6,775.0	6,861.4
Sixth day.....		10.7	1.3	+33.0	6,588.2	6,621.2
Seventh day.....		9.7	2.3	+58.4	6,445.0	6,503.4

^a Computed from the CO₂, assuming a nonprotein respiratory quotient of 0.707.

^b Correction for steer No. 57, 16.86 Cals. per 100 kgm. live weight per hour; for steer No. 60, 6.63 Cals. per hour.

TABLE 12.—Contents of alimentary tract of steers after fast

Steer No.	KILOGRAMS DRY MATTER					
	Contents of—					
	Paunch and reticulum	Omasum	Abomasum	Small intestine	Large intestine	Total, alimentary tract
Steer 57, after 7 days' fast.....	0.378	0	0.028	0.085	0.036	0.527
Steer 60, after 7 days' fast.....	.186	0	.035	.079	.035	.335
KILOGRAMS FRESH SUBSTANCE						
Steer 57, after 7 days' fast.....	10.730	.054	.231	3.696	1.080	15.791
Steer 60, after 7 days' fast.....	16.032	.130	.518	3.083	1.357	31.120

TABLE 13.—Measurements of surface area of steers after fast

Steer No.	Measured area of hide	Computed by Moulton's formula	Computed by Hogan's formula
	<i>Square meters</i>	<i>Square meters</i>	<i>Square meters</i>
57.....	4.774	4.542	4.131
60.....	4.717	4.429	4.324

TABLE 14.—Derivation of values for surface area and maintenance requirement of net energy during the maintenance period preceding fast

Steer No.	Surface area during fast	Fasting katabolism*		Empty weight of fasting animals	Loss of body tissue after maintenance until end of fast	Computed empty weight in maintenance period	Computed surface area in maintenance period	Maintenance requirement of net energy per head
		Total	Per square meter of body surface					
	<i>Sq. meters</i>	<i>Calories</i>	<i>Calories</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Sq. meters</i>	<i>Calories</i>
57.....	4.774	7,838	1,642	363.4	15.4	378.8	4.899	8,044
60.....	4.717	7,034	1,491	333.1	14.4	347.5	4.843	7,221

* Fourth day of fast.

TABLE 15.—Heat production, corrected for differences in live weight

Steer No. and plane of nutrition	Period No.	Average live weight	Correction of heat production to basis of live weight of fast	Heat production			
				Uncorrected for live weight		Corrected to uniform live weight	
				Observed	Computed	Observed	Computed
		<i>Kilograms</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>
Steer 60:							
Fast.....	15	383.0	0				
One-half maintenance.....	11	381.0	+25	7,572	7,564	7,597	7,589
Maintenance.....	1	310.9	+913	7,337	7,227	8,250	8,140
One and a half times maintenance.....	3	332.9	+628	8,963	8,781	9,591	9,409
Two times maintenance.....	5	358.4	+304	11,324	11,143	11,628	11,447
Two and a half times maintenance.....	7	391.8	-107	14,119	14,027	14,012	13,920
Three times maintenance.....	9	426.6	-524		16,224		15,700
Maintenance (alfalfa).....	13		-187	9,916	9,616	9,729	9,429
Steer 57:							
Fast.....	14	399.2	0				
One-half maintenance.....	10	398.3	+12	8,047	8,050	8,059	8,062
Maintenance.....	2	359.6	+527	8,103	8,038	8,630	8,565
One and a half times maintenance.....	4	384.3	+196	9,791	9,591	9,987	9,787
Two times maintenance.....	6	403.1	-51	12,252	12,280	12,201	12,229
Two and a half times maintenance.....	8	443.7	-572	14,678	14,542	14,106	13,970
Maintenance (alfalfa).....	12		-206	10,313	10,046	10,107	9,840

EXPERIMENTAL DATA

The foundation data of the experiments and the computations necessary for their interpretation comprise Tables 2 to 15, inclusive, while the derived final results are to be found in Tables 16 to 22, inclusive. For explanation of the significance of the experimental records and of the methods of computation employed (except for net-energy values) the earlier paper (4) on the subject of the present

discussion should be consulted. The treatment is practically identical in both papers (with the above-mentioned exception), and the arrangement of tables is the same. In the interest of economy of space, therefore, Tables 2 to 15, inclusive, are submitted for permanent record only, without discussion other than the following notes on unusual conditions. A change of attitude in regard to methods of computation of net energy is expressed in the discussion of Table 19, and Figures 3 and 4.

In period 1, with steer No. 60, and period 8 with steer No. 57, the data for the second day's metabolism were vitiated by technical errors. (Table 3.) The values used for the heat production in these periods, therefore, are the averages of the first and the third days' measurements.

It will be observed in Table 7 that the factor expressing the difference in the energy cost of standing as compared with lying is only one-third as large for steer No. 60 as for steer No. 57. No observations were made which explain this difference; but the higher value obtained with steer No. 57 raises a question as to whether this animal was more restless than steer No. 60 while standing, or whether steer No. 60 may not have leaned on the side of the stall for support while standing, during the fasting period, from the results of which this factor was derived. Whatever the significance of these data there was no recourse other than to use them as obtained.

The heat production of fast, as the base value in energy metabolism, enters into nearly all of the computations relating to the utilization of food energy. A change from the previous procedure was made in the treatment of the steers preparatory to the measurement of this value, in that grain alone was given during the last seven days of feeding, the idea being that the withholding of roughage during this interval would lead to a more complete evacuation of the alimentary tract and would make shorter the time required to reach complete fast.

The use of a physic in the preparation of the steers for the measurement of the heat production of fast was the same as in the previous year's work. That is, two doses were given, separated by a 2-hour interval, on each of the two mornings following the last allowance of feed.

In the previous year's study, in which the ration given in the interval immediately preceding fast was hay alone, the dry matter of the contents of the alimentary tract was reduced to 1.737 and 1.127 kgm., after seven and six days' fast, with steers Nos. 36 and 47, respectively; while in the experiments under discussion (Table 12), in which grain alone was given during the last seven days of feeding, the dry matter of the contents of the alimentary tract was reduced, after seven days' fast, to 0.527 and 0.335 kgm., with steers Nos. 57 and 60, respectively. The purpose of this change of procedure, therefore, was accomplished, in that the contents of the alimentary tract—already virtually negligible—were still further reduced.

In determining the heat production of fast it has been the custom at this institute to use for this measure the directly observed heat production, checked by the heat production as computed by the respiratory-quotient method. In the present series of experiments, however, neither of these measurements was entirely satisfactory. The direct measurement was affected by two unusual conditions in the

operation of the calorimeter which seem to have resulted in irregular flow of the cooling water and in unsatisfactory heat absorption. The calorimeter, therefore, appears not to have been in perfect balance during the fasting periods. The respiratory quotients obtained were improperly variable, apparently as a result of storing the air samples wet, and were not in harmony with the prevailing state of fast. Both of the measurements of heat production, therefore, seemed to be unreliable. Fortunately, however, the carbon dioxide measurements appear to have been correct, as shown by the fact that the gravimetric determinations were, on an average, 99.8 and 99.9 per cent of the Sonden determinations, with the two steers. The determinations by the two methods were made on separate aliquots of the air, the gravimetric determination in a continuous sample taken by a motor-driven pump, and the Sonden estimation on a series of intermittent air samples taken at intervals of 15 minutes.

In the light of later results—as yet unpublished—and in consideration of the method of preparation of the steers for the fasting experiments, it was assumed, with confidence, that these animals had reached a status of true fast, with a nonprotein respiratory quotient of approximately 0.707, by the beginning of the fourth day of fast—the first day of the fasting heat measurement—and on this basis the heat production of fast was computed.

The employment of this value as the heat production of fast completes the curves of heat production in relation to the plane of nutrition in a manner harmonious with the curves derived in the previous study, and therefore involves the same conception as to the significance of the fasting heat production, namely, that it comprises two factors, a waste heat of utilization of body nutrients katabolized, and a hypothetical minimum base value of the energy metabolism, including no such energy loss or expense of utilization.

In this light the use of the actual heat production of fast as the minimum base value of energy metabolism would appear to be justified only by the fact that it is measurable, while there is as yet no method known for estimating the lower, hypothetical, minimum value with accuracy.

The problem of standardization of the determination of the fasting heat production as the minimum, measurable base value of energy metabolism is being studied as the major item in the current program of experimentation at this institute.

In Table 8 is given the basis for the computation of the energy equivalent of the nitrogen eliminated per 12-hour subperiod, to be used, as shown in Table 10, in the computation of the total heat production of fast.

In view of the fact that the methods employed in these experiments did not make possible the determination of exact end points in the urinary nitrogen elimination there may be an appreciable error in the quantity given for energy of protein katabolized, and, therefore, a resulting error in the total heat production. A device has since been perfected for giving these desired end points with a high degree of exactness.

In Table 13 are given the measured areas of the removed hides of the experimental subjects, and for comparison the surface areas as computed by the methods of Moulton (6) and of Hogan (5). If the direct measurement of the removed hide is considered to be a correct

measure of the surface area, neither of the computation methods referred to gives nearly enough the same results to be satisfactory for this purpose. It is true that there is opportunity for error in even the direct measurement of the area of the removed hide, but the writers believe that this is the most nearly accurate measure of any thus far proposed.

In Table 15 are given the values of the directly observed and the computed heat production corrected for differences in live weight in order that they may be on a comparable basis. It will be noted that these corrections are in some cases extensive; thus in five experimental periods it was over 500 Calories in extent and varied between a plus correction of 913 Calories and a minus correction of 572 Calories. It is unfortunate that such extensive corrections of the heat production as observed, were necessary.

HEAT PRODUCTION IN RELATION TO THE PLANE OF NUTRITION

The observed and the computed heat production, corrected as in Table 15, are averaged and presented in Table 16, and are graphically shown in Figure 1 plotted against the dry matter of the feed eaten. (Table 2, or 5, or 17.)

TABLE 16.—Average of observed and computed heat production, corrected for differences in live weight, and heat increments derived by comparison of the heat production in the periods of feeding with that of fast

Steer No. and period	Plane of nutrition	Heat production	Fasting katabolism	Heat increments	
				Total	Per kilogram of dry matter
Steer 60:		Calories	Calories	Calories	Calories
Period 11.....	One-half maintenance.....	7,593	7,034	559	333
Period 1.....	Maintenance.....	8,195	7,034	1,161	411
Period 3.....	One and a half times maintenance.....	9,500	7,034	2,466	582
Period 5.....	Two times maintenance.....	11,538	7,034	4,504	730
Period 7.....	Two and a half times maintenance.....	13,966	7,034	6,932	922
Period 9.....	Three times maintenance.....	15,700	7,034	8,666	913
Period 13.....	Maintenance (alfalfa hay only).....	9,579	7,034	2,545	508
Steer 57:					
Period 10.....	One-half maintenance.....	8,061	7,838	223	131
Period 2.....	Maintenance.....	8,598	7,838	760	246
Period 4.....	One and a half times maintenance.....	9,387	7,838	2,049	444
Period 6.....	Two times maintenance.....	12,215	7,838	4,377	702
Period 8.....	Two and a half times maintenance.....	14,038	7,838	6,200	770
Period 12.....	Maintenance (alfalfa hay only).....	9,974	7,838	2,136	414

TABLE 17.—Partition of energy of feed

Steer No. and period	Dry matter of feed mixture	Energy per kilogram of dry matter							
		Gross	Digestible	Metabolizable	Total net	Feces	Heat increment	Methane	Urine
Steer 60:	Kgm.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.
Period 11.....	1,681	4,468	3,202	2,540	2,207	1,266	333	439	222
Period 1.....	2,828	4,468	3,242	2,668	2,267	1,224	411	375	199
Period 3.....	4,237	4,468	3,178	2,668	2,071	1,290	582	350	175
Period 5.....	5,704	4,468	3,156	2,613	1,823	1,312	790	366	178
Period 7.....	7,520	4,468	3,094	2,589	1,667	1,375	922	335	170
Period 9.....	9,489	4,471	3,000	2,518	1,605	1,471	913	324	158
Steer 57:									
Period 10.....	1,700	4,468	3,249	2,584	2,453	1,219	131	438	227
Period 2.....	3,085	4,466	3,286	2,672	2,426	1,180	246	412	202
Period 4.....	4,612	4,468	3,279	2,704	2,260	1,189	444	368	190
Period 6.....	6,233	4,468	3,180	2,625	1,923	1,288	702	375	180
Period 8.....	8,057	4,471	3,121	2,588	1,818	1,350	770	364	169

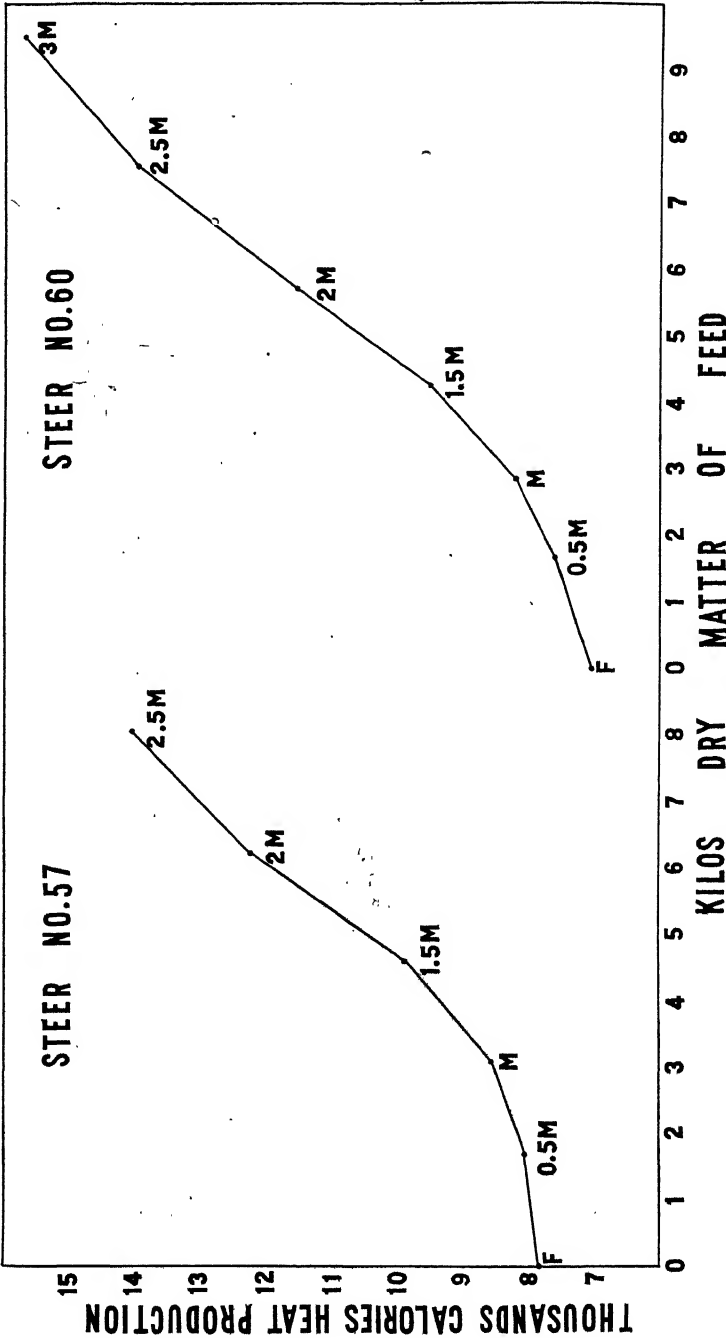


FIGURE 1.—Heat production as affected by the plane of nutrition of cattle

The curves representing this relationship confirm all details revealed in the earlier study of this subject; and add to the earlier presentation by extending the feeding with steer No. 57 to the plane of two and one-half times the maintenance requirement, and with steer No. 60 to three times maintenance—these extensions of the evidence continuing the tendencies manifest in the curves representing the earlier study.

This confirmation of the earlier findings is made especially impressive by the fact that the sequence of the experimental treatments (Table 1) was quite different from that in the earlier study, thus showing that the apparent relationship of the heat production to the plane of nutrition is real, and that the character of the curves representing this relationship is not determined, to any large extent, by a carrying over of an influence from one period to that which follows in the experimental program.

The curves representing the two animals are of identical significance. With the heat production of the fourth day of fast as the base value, the heat production increased slowly between fast and maintenance, and much more rapidly above maintenance, but with a decreased rate of rise between the planes of two and three times the maintenance requirement.

With largely the same understanding as expressed in the earlier paper (4), and with the conception as to the composition of the heat production of fast given on page 51, the curvature of the line of heat production in relation to increasing food consumption is interpreted as resulting from (1) the increasing concentration of metabolites circulating in the blood; (2) the change in the proportions of protein, fat, and carbohydrate katabolized, with increase in the katabolism of food nutrients and decrease in the katabolism of body nutrients; (3) the energy expense of synthesis of body nutrients (fat from carbohydrates); and (4) the decreased metabolizability of the food at the higher planes of nutrition—on account of which the quantities of food available for the use of the animal are not quite as great, proportionately, as the quantities eaten.

This assumes a difference in the energy expense of utilization of protein, carbohydrate, and fat in the metabolism of cattle. This assumption is made on the strength of the well-known difference in the specific dynamic effects of the three general classes of nutrients with other species; but with the understanding that on account of the heat of fermentation of carbohydrates, at least, if for no other reason, the relation of the energy expense of utilization of protein, carbohydrate, and fat in the metabolism of cattle must be different from that which characterizes the metabolism of the nonruminants in which this factor has been investigated.

The most interesting part of the curve of heat production is that between fast and maintenance, which expresses the resultant of the influence of body nutrients and of food nutrients metabolized.

A difficult conception necessary to the appreciation of the significance of this curve is that of the theoretical, minimum, base value of energy metabolism (referred to on p. 53)—the energy requirement of the animal living entirely on its own tissues, at 100 per cent efficiency of transformation of their metabolizable energy into vital energy; the heat production representing the actual energy need; there being complete oxidation of metabolizable nutrients, with no waste of heat in transformation, or through stimulation by metabolites; the organ-

ism existing, therefore, in a manner comparable to that of an internal-combustion engine operating under such hypothetical conditions that the work done would be equivalent to the potential energy of the fuel.

The difference between this minimum energy requirement of life and the heat production of the immobile animal during fast is the assumed energy expense, or waste, of utilization of the energy of the body tissue katabolized.

It is the magnitude of this observed heat production of fast which determines the lowest point, or base value, in this curve of heat production, and merely by so doing contributes largely to the determination of this curve between fast and maintenance.

For purposes of study and comparison similar curves of heat production as related to the metabolizable energy instead of the dry matter of the rations were drawn. These, however, are not presented, since, in view of the facts that energy is expended in the handling of the nonmetabolizable portion of the food, and that there is a closer relation between the dry matter and the heat production than between the metabolizable energy and the heat production, the dry matter is in reality a more significant base of reference than is the metabolizable energy.

This statement is based in part on the coefficients of correlation for the values entering into Figure 4. Thus the coefficients of correlation of the metabolizable energy and the heat production of mixed rations is 0.732 ± 0.026 , and of the roughages is 0.76 ± 0.039 ; while the corresponding values for dry matter and heat production are, for mixed rations, 0.905 ± 0.011 , and for roughages, 0.863 ± 0.015 .

The curve of heat production in relation to the metabolizable energy is of the same character as the curve of heat in relation to the dry matter, though the degrees of curvature are somewhat less.

The decreased rate of rise in heat production at the highest planes of nutrition, which is to be seen in the curves based on the dry matter, is also present, though in reduced degree, in the curves based on the metabolizable energy.

ENERGY EXPENDITURE OF FOOD UTILIZATION

The net energy of a ration is the gross energy minus the energy of the excreta—urine, feces, and methane (which can be determined directly) and the energy expense of food utilization (the heat increment) which must be determined as the difference in heat production, as observed in two experimental periods, related to the concurrent difference in food.

In consideration of the fact that the quantitative relationship of the heat production to the food consumption at different planes of nutrition is such as to be expressed not by a straight line but by a reversed or S curve, it is obvious that the heat increase per unit of food, that is, the heat-increment value of the food, must differ with the plane of nutrition; and that the influence of the heat increment in the determination of net-energy values is such as to lead to a different net-energy value at each different plane of nutrition, though this influence is modified by the other factors which enter into the determination of net energy. Moreover, the conceptions of the significance and composition of the heat production of fast, as set forth on page 53, and of the factors which determine the curve of heat production in relation to

feed consumption, as stated on page 56, imply differences in the significance of heat increments above and below maintenance, especially in that heat increments between planes of nutrition below maintenance can not be considered as expressing only the energy expense of food utilization, as do heat increments above maintenance, but may be regarded as affected by the factor of waste heat of utilization of body nutrients.

The problem presented by the heat increment in relation to the determination of net-energy values is as to how these values shall be derived and how they shall be treated in order that they may contribute to the determination of net-energy values of the greatest practical usefulness.

In the light of the foregoing observations it will be understood that in a literal sense a net-energy value is exactly true only at the plane of nutrition at which it has been determined; but since it would be a hopeless task to derive such a series of sliding scales of net-energy values as is suggested by this fact, some arbitrary simplification must be adopted if the net-energy conception is to lead to any practicable system of measures in nutrition. With this desideratum in mind, therefore, the heat increments derived from these experiments will be considered.

The heat-increment values of the rations fed, based on the heat production of fast, are given in Table 16 arranged in order of rising planes of nutrition. All of these increments except the last, with each steer, apply to the mixed ration of hay and grain; the last applies to the ration of alfalfa hay alone.

If the heat production were a rectilinear function of the food energy it is obvious that the heat increments of the mixed ration, at the several planes of nutrition, however computed, would be alike; but in harmony with the observed relationship of the heat production to the plane of nutrition (Table 16 and fig. 1) the heat increments of the mixed ration, as computed with reference to the heat production of fast (Table 16) are characterized by such marked individuality that they could not consistently be averaged for any purpose. The increment between fast and maintenance, however, has a special significance as the energy expense of utilization of food for the preservation of energy equilibrium.

TABLE 18.—*Net-energy values for maintenance*

Period No.	Steer No.	Dry matter of feed eaten			Total heat production	Fasting katabolism	Heat increments		Metabolizable energy per kilogram of dry matter	Net energy per kilogram of dry matter (for maintenance)	Utilization of metabolizable energy (for maintenance)
		Alfalfa hay	Corn meal	Total			Total	Per kilogram of dry matter			
		<i>Kgm.</i>	<i>Kgm.</i>	<i>Kgm.</i>	<i>Cals.*</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Per cent</i>
13.....	60	5.013	-----	5.013	9,579	7,084	2,545	508	1,927	1,419	73.6
12.....	57	5.155	-----	5.155	9,974	7,838	2,136	414	1,976	1,562	79.0
1.....	60	1.420	1.408	2.828	8,195	7,084	1,161	411	2,668	2,257	84.6
2.....	57	1.546	1.539	3.085	8,598	7,838	760	246	2,672	2,426	90.8
Computed values for corn meal.	60	-----	-----	-----	-----	-----	-----	313	3,415	3,102	90.8
Do.....	57	-----	-----	-----	-----	-----	-----	78	3,371	3,293	97.7

* Average of the observed and computed heat production corrected for difference in live weight.

TABLE 19.—*Net-energy values for body increase, the values for the individual feeds being computed by the heat-increment-proportional method, and by the net-proportional method*

Steer No.	Periods compared	Ration	Planes of nutrition compared	Metabo- lizable energy per kilo- gram of dry matter	Values by heat-increment- proportional method				Values by net proportional method			
					Heat in- crement per kilo- gram of dry matter	Net energy per kilo- gram of dry matter	Utiliza- tion of metabo- lizable energy	Per cent	Heat in- crement per kilo- gram of dry matter	Net energy per kilo- gram of dry matter	Utiliza- tion of metabo- lizable energy	Per cent
60	1 and 3	Hay and meal	Maintenance and one and one-half times maintenance	2,653	926	1,727	65.1	65.1	926	1,727	65.1	65.1
57	2 and 4	do	do	2,704	844	1,860	68.8	68.8	844	1,860	68.8	68.8
60	1 and 5	do	Maintenance and two times maintenance	2,613	1,162	1,451	55.5	55.5	1,162	1,451	55.5	55.5
57	2 and 6	do	do	2,625	1,149	1,476	56.2	56.2	1,149	1,476	56.2	56.2
60	1 and 7	do	Maintenance and two and one-half times maintenance	2,389	1,230	1,359	52.5	52.5	1,230	1,359	52.5	52.5
57	2 and 8	do	do	2,388	1,094	1,494	57.7	57.7	1,094	1,494	57.7	57.7
60	1 and 9	do	Maintenance and three times maintenance	2,318	1,127	1,391	55.2	55.2	1,127	1,391	55.2	55.2
57	2 and 3	Alfalfa hay	Maintenance and one and one-half times maintenance	1,016	1,145	771	40.2	40.2	630	1,301	55.2	55.2
60	1 and 4	do	Maintenance and two times maintenance	2,000	1,420	580	29.0	29.0	802	1,086	59.9	59.9
57	2 and 5	do	do	1,837	1,436	451	23.9	23.9	975	1,198	48.3	48.3
60	1 and 6	do	Maintenance and two and one-half times maintenance	1,941	1,320	7	0.4	0.4	991	950	45.7	45.7
57	2 and 7	do	do	1,870	1,320	350	18.7	18.7	1,018	854	50.3	50.3
60	1 and 8	do	Maintenance and three times maintenance	1,914	1,841	73	3.8	3.8	952	962	48.0	48.0
57	2 and 9	do	do	1,819	1,383	426	23.4	23.4	945	874	69.9	69.9
60	1 and 3	Con meal	Maintenance and one and one-half times maintenance	3,396	705	2,691	79.2	79.2	1,023	2,373	73.1	73.1
57	2 and 4	do	do	3,411	298	3,143	92.1	92.1	1,885	2,526	73.6	73.6
60	1 and 5	do	Maintenance and two times maintenance	3,345	885	2,460	73.5	73.5	1,350	1,995	69.4	69.4
57	2 and 6	do	do	3,312	364	2,948	89.0	89.0	1,310	2,002	69.3	69.3
60	1 and 7	do	Maintenance and two and one-half times maintenance	3,314	937	2,377	71.7	71.7	1,337	1,867	69.3	69.3
57	2 and 8	do	do	3,265	347	2,918	89.4	89.4	1,247	2,028	69.3	69.3
60	1 and 9	do	Maintenance and three times maintenance	3,223	858	2,365	73.4	73.4	1,312	1,911	59.3	59.3

* Derived from supermaintenance periods (nos. 3-9) only.

A comparison of these heat increments, computed with reference to fast, with increments based on the heat production of maintenance, as in Table 19, shows that the latter are the higher values; thus, the heat-increment values between maintenance and two times maintenance are 1,149 and 1,162 Calories, while the increments between fast and two times maintenance are 702 and 790 Calories, respectively, for the two steers.

The difference in the heat increments computed on these two bases calls attention to the fact that it is the magnitude of the heat production of fast which is mainly responsible for the low heat-increment and high net-energy values for maintenance.

In the study of the supermaintenance heat increments, those between maintenance and planes of nutrition supplying multiples of the maintenance requirement seem to be convenient units in which to express and to consider the energy expense of utilization of food for body increase.

In Table 20 are presented the heat increments computed from the heat production of consecutive periods of nutrition. This method of computation ordinarily results in the maximum diversity of heat increments, since the differences in food are smaller than when each period is compared with one base value, while the inevitable element of error in heat measurements applies, in any case, to two such evaluations.

TABLE 20.—Heat increments as computed from differences in the heat production of consecutive planes of nutrition

Steer No.	Periods compared	Planes of nutrition compared	Heat increments per kilogram of dry matter
			Calories
60	15 and 11.....	Fast and one-half maintenance	333
57	14 and 10.....	do.....	131
60	11 and 1.....	One-half maintenance and maintenance.....	525
57	10 and 2.....	do.....	388
60	1 and 5.....	Maintenance and one and one-half times maintenance.....	920
57	2 and 4.....	do.....	844
60	3 and 6.....	One and one-half maintenance and two times maintenance.....	1,380
57	4 and 8.....	do.....	1,436
60	5 and 7.....	Two times maintenance and two and one-half times maintenance.....	1,937
57	6 and 8.....	do.....	999
60	7 and 9.....	Two and one-half times maintenance and three times maintenance.....	881

A comparison of these heat increments with those computed with reference to fast shows that the former are the higher values in all cases, except between the lowest two planes of nutrition, where the two methods of computation become one and the same.

The increments between fast and half maintenance are very low. With further rise in the plane of nutrition the heat increments increase rapidly to several times the values between fast and half maintenance; but, when computed from consecutive planes of nutrition, the increments at planes of nutrition above two times maintenance are lower than the maximum.

Obviously the influence of these extremely diverse heat increments on the derived net-energy values is prominent, but is modified by the values for the energy of the excreta—urine, feces, and methane—which also are involved.

A comparison of the heat increments for the two steers, as these values are computed by the three foregoing methods, shows that when based on the heat production of fast (Table 16) the heat increments for steer No. 57 are lower than those for steer No. 60, this difference appearing to be due especially to the high value for the fasting katabolism of steer No. 57. When based on the heat production of maintenance (Table 19), however, all of the increments of the mixed ration for steer No. 60 are the higher; as also are all but one of the increments as computed between consecutive periods. (Table 20.)

These comparisons suggest imperfect management of the calorimeter, or unsuccessful management of the animals with respect to treatment preparatory to fast, or different response of the animals to the fasting treatment, rather than that the energy expense of utilization of the food by steer No. 57 was lower than by steer No. 60, below the maintenance level of nutrition.

In the earlier study the corresponding heat increments for the mixed ration as determined with the two steers agreed very well; but the heat increments as determined in the present series of studies failed to agree, as representing the two animals, or in comparison with those determined the year before.

The study of these heat increments has not resulted in any entirely new conclusions, but has thrown new light upon the understanding derived from earlier studies, and has emphasized the importance of the following considerations:

- (1) The necessity of rigidly standardizing the determination of the heat production of fast as the base value in the study of energy metabolism.

- (2) The necessity of improving the basis for correcting the heat production, as observed, on account of differences in the live weight of the experimental subject in periods the heat production of which is to be compared. (See p. 39.)

- (3) The necessity of determining separate net-energy values at the planes of nutrition of greatest significance, to wit, at maintenance, and at planes of nutrition at which food supplying multiples of the maintenance requirement of energy is fed.

PARTITION OF FEED ENERGY

The partition of the gross energy of the feed into its seven components—from the point of view of this study—is recorded in Table 17 and is graphically exhibited in Figure 2.

As in the previous year's work, the digestible energy increased slightly, with both animals, with increase of food from the half-maintenance to the maintenance level; and, viewing the two years' work together, it seems that this increase is due mainly to crude fiber, but partly to protein; and that ether extract and nitrogen-free extract do not contribute thereto.

With further increase in feed, from maintenance to the highest levels of nutrition, there was, with both steers, a slight but consistently continuous drop in digestible energy, this decrease, as in the previous year's work, being due mainly to nitrogen-free extract and protein.

The increased apparent digestibility of the protein of the ration from the half-maintenance to the maintenance level may be merely

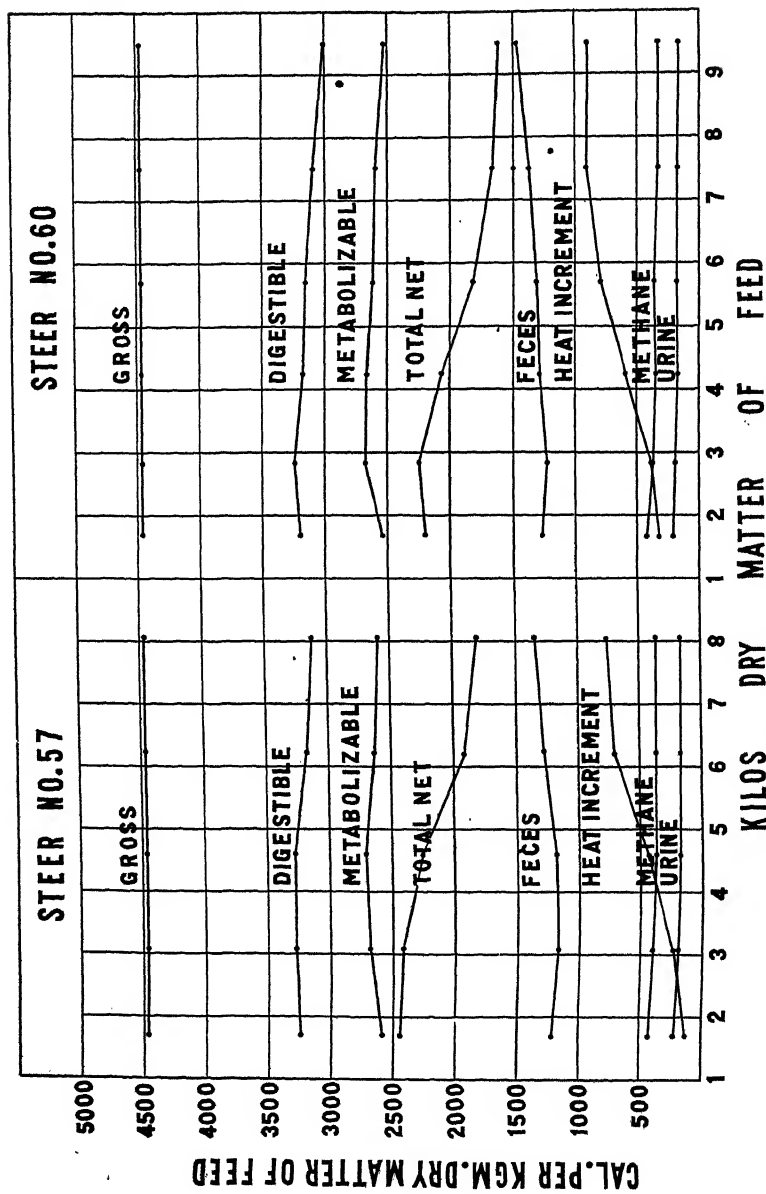


FIGURE 2.—Partition of feed energy as influenced by the plane of nutrition of cattle

an arithmetical result of a decrease in the proportion of metabolic to total protein in the feces; and the coincident increase in the digestibility of crude fiber may be a result of a decrease in some product of methane fermentation which (product) has the effect of limiting the digestibility of crude fiber at the half-maintenance as compared with the maintenance level—the methane fermentation having been found to be less, per unit of feed, during maintenance than during half-maintenance feeding; or the appearance of increased digestibility of both protein and crude fiber at maintenance as compared with half-maintenance planes of nutrition may possibly result merely from a carrying over of fecal residues from preceding high planes of feeding into the half-maintenance periods, though no direct evidence was observed in support of this suggestion.

The metabolizable energy, being the energy of the digestible nutrient minus that of the urine and the methane, follows closely the digestible, in its relation to the gross energy, but is an appreciably lower value than that representing digestible nutriment.

The metabolizable energy is almost exactly the same proportion of the gross energy at the highest and the lowest planes of nutrition, but at intermediate points is higher, the maximum being reached with one steer at the plane of half-maintenance, and with the other at half more than maintenance. The variation of the metabolizable energy in the previous study was closely similar.

The feces curve requires no comment, since it was the reverse of the curve of digestibility, which has been discussed.

The total heat increment (computed with reference to fast) increases with each rise in plane of nutrition, but with a decreasing rate of rise at the highest planes. From lowest to highest planes there is a prominent increase in the heat increment.

The urine and methane values are both small but appreciable in magnitude. They vary little throughout the several planes of nutrition; but decrease slightly, and with a close approach to regularity, from the lowest to the highest plane.

The total net energy—that is, the net for maintenance and body increase together—being the gross energy minus the energy of the urine, feces, methane, and heat increment, is expressed as a curve which is the resultant of the four curves representing the above-mentioned losses and expenses of food utilization.

From the lowest to the highest plane of nutrition there is a general and decided decrease in the net-energy value of the ration, at a rate approaching uniformity, but not being so nearly uniform as in the previous study.

In view of the fact that the primary purpose of net-energy values is to serve as convenient guides in feeding practice, it is obvious that the attainment of this objective requires the radical simplification of the truth, as expressed by these curves of total net energy, and that the method of accomplishing this simplification must be standardized in a conventional manner.

THE PROBLEM OF DETERMINATION OF NET-ENERGY VALUES OF RATIONS AND OF INDIVIDUAL FOODSTUFFS

It is axiomatic that the method of an investigation should reduce the problem to be solved to a single variant, a principle which, in relation to the present problem, involves the extremely exacting im-

plication that during a net-energy determination nutrients must be present of such kinds, quantities, and proportions as completely to satisfy all of the nutritive requirements of the animal for other than energy production, except as the nutrient reserves of the animal body protect it from deficiencies during the comparatively short interval of time covered by the determination; otherwise the energy metabolism must be affected.

With reference to this function the writers have to suggest only that, in the nature of the case, the detailed facts as to the length of time during which the nutritive reserves of the animal body protect it from the deficiencies of rations must vary, as determined by the status of these nutrient reserves, by the intimate character of the rations fed, and by the functional activities of the animal, in an exceedingly complicated manner.

In the routine practice of this institute the standard minimum time employed in a net-energy determination with cattle is 28 days—10 days in which to adjust the animal to the ration and 18 days for the determination of metabolizable energy. The last 3 of these 18 days are utilized also for the measurement of the heat production and respiratory products. It is, of course, impossible to say, in any particular case, how perfectly the nutritive reserves of the animal protect it, during this interval of 28 days, or during a series of such intervals, from shortage of nutrients in the rations fed.

As the net-energy conception antedates, in its origin, much of our present understanding of the details of nutrition, the recent advance in knowledge of the proteins, the vitamins, and the mineral nutrients has led to an understanding of the requirements of net-energy determinations as of continuously increasing complexity. While the principle of net energy is as simple and as irrefutable, therefore, as that of the bank balance, with which it is analogous, an actual net-energy determination really involves all of the intricacies of animal nutrition.

Prominent among the complications of this subject are those resulting from the relationship of the heat production to the plane of nutrition. Since this relationship is such as to be expressed by a reversed or S curve, its effect, as has been observed, is to determine that there are, fundamentally, as many net-energy values of a ration as there are points of observation as to the plane of nutrition—except as the above-mentioned curve of heat production happens to intersect a straight line representing a constant relationship of heat production to food consumed.

On this account the most practicable method of determination and use of net-energy values seems to be as representing definite intervals—as to planes of nutrition of special significance. For instance, a net-energy value determined at the level of energy equilibrium is highly significant, since it expresses the value of the ration for the definite purpose of maintenance; and such maintenance values possess a further significance in that they appear to be at least virtually the same as, if not quite identical with, net-energy values of feeds for milk production.

Naturally, the practical man has no interest in net-energy values applying to any plane of nutrition below maintenance.

Net-energy values for body increase may be determined at planes of nutrition arbitrarily fixed as requiring multiples of the main-

tenance requirement. The plane of twice maintenance seems to be especially significant in that it represents, in a general way, the average use of food, under conditions of practice. It is true that this is only a moderate rate of feeding; that full feed varies greatly with the character of the ration; that animals sometimes eat much more than twice maintenance; and that no one plane of nutrition is definitely representative of feeding for body increase. However, if determinations of net-energy values for body increase are to be made, as a standard procedure, at some one plane of nutrition—as the writers consider desirable—this must be at a plane at which the experimental animals can be depended on to eat up clean, without waste or refusal of parts, the more important and the greater number, at least, of the practical feeds; and to meet these requirements twice maintenance is provisionally adopted as a representative level of feeding for body increase. For special purposes, net-energy values might be determined at higher planes of nutrition.

The supplementing effects of foodstuffs on each other, as they are fed together, are also important in relation to the determination of net energy, since the net-energy values of nearly all feeds must be determined as the difference between results obtained with a particular ration and with the same plus the food product of interest. In such computations the entire supplementing effect of the combination is attributed to that component of the ration which is treated as the supplement, whereas, logically, it should in some way be apportioned between the basal portion of the ration and the supplement, as becomes obvious when a very small quantity of the supplement comes to be credited, by virtue of its apparent capacity to add to the effectiveness of the basal ration, with an absurd valuation.

The smaller the proportion of the supplement which is involved, the larger will be the unit effect of this influence. As an instance of this fact, it was shown by the senior author many years ago (2) in feeding experiments with growing swine, to which linseed meal and wheat middlings were fed in different proportions as supplements to corn, that the smaller the proportion of the supplement fed, and consequently the more deficient the ration in protein, the less efficient was the ration as a whole, as determined by the gain in weight in relation to feed eaten; but the greater was the supplementing, or corn-replacement value, of the protein supplements per unit of their own weight; and, we now understand, the greater must have been their net-energy values.

In the light of these facts it may be that there are, in reality, different net-energy values of feeding stuffs—for growing animals at least—in accord with each different nutritive ratio in which the feeding stuffs are combined. If such is the case, net-energy values should be determined in rations so compounded either that their digestible nutrients are contained in the optimum nutritive ratio, or that they represent especially significant conditions of practice.

It is also true that an accurate method has not yet been derived for computing the heat production, as observed in different experimental periods, and with the animal at different live weights, to the basis of the same live weight (see p. 39). Obviously it is important to improve the basis for this correction; and this is entirely practicable, but will require extensive studies, especially of the influence of

the plane of nutrition on the content of feeds and feed residues and on the heat of fermentation of carbohydrates in the alimentary tract.

Furthermore, there are serious arithmetical contributions to the difficulties of determining consistent net-energy values of foods. The most important of these derive from the fact that net energy can not be determined directly but is determined by difference—as a remainder, in the simplest case, after subtracting from the gross energy of the food the energy represented by (1) the urine, (2) the feces, (3) the methane produced, and (4) the energy expense of food utilization—measured as the difference between the heat production at two planes of nutrition, related to the coincident difference in food—each of these four losses and expenses of utilization varying in a different way, as related to the plane of nutrition. The total of errors of work, whether additive or compensating, are obviously thrown into the remainder in the computation not only of the heat increment but also in the final computation of the net energy, as above outlined.

An idea of the mass of data affected by this principle may be derived from the fact that in a single 3-day balance of matter and energy, two of which are involved in the determination of one heat increment, as in the procedure outlined above, about 10,000 observations of one kind or another are made. It is true that the greater part of these observations are made in connection with the direct measurement of heat production, and that the number could be greatly reduced by determining the heat production by the respiratory-quotient procedure; but in the work of this institute, thus far, the direct measurement has been the more certain and accurate.

There is but one method, according to the procedures of this institute, for determining the net-energy value of a grain and of a roughage for maintenance. By this method the metabolizable energy of the roughage is determined directly from the feeding of a maintenance ration of roughage alone; the heat increment of the roughage is measured as the difference between the heat production of fast, and of maintenance on roughage alone; and the net energy is the difference between the metabolizable energy and the heat increment. Then the net-energy value of the grain is determined from the net-energy value of a mixed ration of grain and roughage (determined as above), by assuming the net-energy value of the roughage in this mixed ration to be the same as when the roughage is fed alone, the remainder being the net energy of the grain in the mixed ration.

This procedure has two weaknesses: The total effect of combining the grain and the roughage, and the resultant of all errors of work are assigned, by subtraction, to the grain alone.

Feed used for maintenance, however, is in an important sense a total loss, and the feeder's immediate interest is in the value of feeds for purposes of production after the animal's maintenance has been provided for. Very few individual feeds can be fed alone at practical rates of production; almost all feeds require to be fed as mixed rations; and one of the greatest difficulties in the application of the net-energy conception to the measurement of nutritive values of feeds is that of apportioning the net-energy value of a mixed ration—fed at a plane of production—between the components of such a ration, especially between the grain and the roughage.

For the attainment of this objective, that is, for the determination of separate net-energy values of a grain and a roughage, for body increase, three methods will be discussed (1) the feed-and-plane-difference method, (2) the net-proportional method, and (3) the heat-increment-proportional method. Each of these methods requires three experimental periods, one on a ration of roughage alone, at maintenance; a second on a ration of grain and roughage, at maintenance; and a third on a ration of grain and roughage, at the production level.

According to the feed-and-plane-difference method the net-energy value of the grain, for production, is determined by comparison of a ration of roughage, at maintenance, with a ration of grain and roughage, at the production level; while the net-energy value of the roughage, for production, is determined by a comparison of a ration of grain and roughage, at maintenance, with a ration of grain and a larger proportion of roughage, at the production level. This method involves, therefore, comparisons of rations differing not only in quantity but also in kind, and unproven assumptions (1) that the metabolizable-energy value of the roughage in the mixed ration at the production level is the same as that of the roughage when fed alone at the maintenance level, and (2) that the metabolizable-energy value of the grain is the same in the two mixed rations, of different composition, at the maintenance and the production levels.

In other words, this method involves any such errors as may result from the assignment—to whichever component (grain or roughage) is treated as the supplement—of the effects (on heat-increment value or metabolizable-energy value) of the difference in the planes of nutrition of the rations compared, and of the combination of the components of the mixed rations.

It is necessary, in order to minimize, as much as practicable, the supplementing effects just mentioned, to combine grain and roughage with due consideration of the proportions in which they would be fed in practice, especially in view of the fact that the smaller the proportion in which the supplement is fed, in the mixed ration, the greater will be the supplementing effect per unit of its own substance, and, consequently, the greater the distortion of its most significant net-energy value. Table 21 is presented as an illustration of this method of computation, the value derived in this case, on the basis of assumed data, being the net-energy value of grain for production.

TABLE 21.—*Example of the feed-and-plane-difference method*

Period No.	Dry matter of feed		Metabolizable energy	Heat production
	Roughage	Grain		
	<i>Kilograms</i>	<i>Kilograms</i>	<i>Calories</i>	<i>Calories</i>
1.....	5	2.5	18,000	12,625
2.....	5	None.	9,625	9,500
Difference equals.....	0	2.5	8,375	3,125
Difference per kilogram of grain equals.....			3,350	1,250

The net energy per kilogram dry matter of grain=3,350-1,250=2,100 Calories.

According to the net-proportional method the determination of the separate net-energy values of a grain and a roughage for production

requires (1) a determination of the net-energy value of a mixed ration of the grain and the roughage, for production, by a comparison of such a ration at the production level with one of the same composition fed at the maintenance level, and (2) a computation of the net-energy values of the individual feeds, for production, based on the assumption that these values of the grain and the roughage for production are to their net-energy values for maintenance as the net-energy value of the mixed ration for production is to the net-energy value of the same for maintenance.

As an illustration of the net-proportional method the following is presented:

The net-energy value of the mixed ration of alfalfa hay and corn meal for maintenance as computed in Table 18, for steer No. 60, is 2,257 Calories per kilogram of dry matter. The net-energy value for body increase of the same mixture of alfalfa hay and corn, fed at a level of twice the maintenance requirement, as computed in Table 19, is 1,451 Calories per kilogram of dry matter. The ratio of net for body increase to net for maintenance is as 1,451:2,257, or as 0.643:1.

The net-energy values of alfalfa hay and of corn meal for maintenance of steer No. 60 are, as shown in Table 18, 1,419 Calories and 3,102 Calories per kilogram of dry matter, respectively. Hence, the individual net-energy values of alfalfa hay and of corn meal for body increase, at the two-times-maintenance level, are 912 ($1,419 \times 0.643$) Calories and 1,995 ($3,102 \times 0.643$) Calories per kilogram of dry matter, respectively. (Table 19.)

The fundamental assumption on which this method is based seems to the writers to be of doubtful validity, since it deals with the net energy as though it were of direct significance, whereas, as has been explained (p. 66), it is only a remainder, the character of which is determined as the difference between the gross energy of the feed, as the minuend, and the sum of the quotas of energy representing (1) the urine, (2) the feces, (3) the methane, and (4) the energy expense of food utilization, as the subtrahend. But whatever element of inconsistency there may be in the net-proportional method it seems unlikely that gross distortion of the true net energy values of individual feeds could result from the employment of this method since they are computed from the ratio of the net-energy value of a mixed ration for maintenance to the corresponding value for production, and since this ratio is not far from 1.

The heat-increment-proportional method requires the same number of experimental periods, with the same treatments, as are required by the net-proportional method—the computation of the individual net-energy values of grain and roughage for production depending on the assumption that their heat-increment and metabolizable-energy values for production are to their heat-increment and metabolizable-energy values for maintenance as the heat-increment and metabolizable-energy values of the combined ration of grain and roughage for production are to the heat-increment and metabolizable-energy values of the same for maintenance.

This assumption seems plausible, but is without basis of established fact and is characterized by serious practical shortcomings.

Thus, in computing the heat-increment value and the metabolizable-energy value of the grain and of the roughage for maintenance to

such values for body increase, the heat-increment values are multiplied by factors which may be several units in magnitude, while the metabolizable-energy values are multiplied by factors quite near to unity.

Such elements of error as there may be in the individual metabolizable-energy values of grain and of roughage, for maintenance, therefore, are not much altered in extent in the process of translation into values for body increase; but the errors in the heat-increment values of grain and of roughage, for maintenance, may be multiplied several-fold in being translated into values for body increase; and these exaggerated errors of heat-increment value may serve prominently to distort net-energy values, since they apply directly to the net, by subtraction.

Furthermore, since net-energy values of feeds for body increase are of lesser magnitude than are net-energy values for maintenance, the multiplied errors of translation of heat increment and metabolizable energy for maintenance to such values for body increase gain further power to distort net-energy values of feeds for body increase through this diminution of the base (net energy) affected.

Again, an error of any sort which affects the heat-increment or metabolizable-energy value of the mixed ration at either the maintenance or the production level thereby affects the factor for translation of maintenance values into production values, and so affects net-energy values for production.

As an illustration of the difference in the possibilities of distortion of heat-increment values and metabolizable-energy values, through multiplication of the element of error, by the net-proportional as compared with the heat-increment-proportional method, the following is presented:

In computing the heat-increment value of the mixed ration, for maintenance, in these experiments, to such a value at the two-times-maintenance level, by the heat-increment-proportional method, it is necessary (Tables 18 and 19) to multiply by the factors 2.83 ($1,163 \div 411$) and 4.67 ($1,149 \div 246$) for the two steers; while in the use of the net-proportional method the maintenance values of net energy are computed to production values at the two-times-maintenance level by the use of the factors 0.64 ($1,451 \div 2,257$) and 0.61 ($1,476 \div 2,426$) for the two steers.

As an example of the heat-increment-proportional method the following is presented.

The heat-increment value of the mixed ration of alfalfa hay and corn meal for maintenance of steer No. 60 is 411 Calories. (Table 18.) The heat-increment value of the mixed ration for body increase at the two-times-maintenance level is 1,162 Calories. The ratio of heat increment for body increase to heat increment for maintenance is as 1,162:411, or as 2.827:1.

The heat-increment values of alfalfa hay and of corn meal for maintenance of steer No. 60 are 508 Calories and 313 Calories, respectively. (Table 18.) Hence the heat-increment values of alfalfa hay and of corn meal for body increase, at the two-times-maintenance level, are 1,436 (508×2.827) Calories and 885 (313×2.827) Calories, respectively. (Table 19.)

The ratio of the metabolizable energy per kilogram of dry matter of the mixed ration at the level of two times maintenance to the metabo-

lizable-energy value of the same at the maintenance level (Tables 18 and 19) is as 2,613:2,668, or as 0.9794:1.

The metabolizable-energy values of alfalfa hay and of corn meal for steer No. 60 at the maintenance level are 1,927 Calories and 3,415 Calories, respectively. (Table 18.) Hence, the metabolizable-energy values of alfalfa hay and of corn meal at the two-times-maintenance level are 1,887 ($1,927 \times 0.9794$) Calories, and 3,345 ($3,415 \times 0.9794$) Calories, respectively. (Table 19.)

The net-energy values of alfalfa hay and of corn meal, for body increase at the two-times-maintenance level, are, therefore, 451 ($1,887 - 1,436$) Calories and 2,460 ($3,345 - 885$) Calories, respectively. (Table 19.)

The net-energy values which were determined in the present study will now be considered, those for maintenance being given in Table 18.

These figures for the mixed ration, for the alfalfa hay, and for the corn meal agree satisfactorily with the corresponding values as determined in the previous study—as they should, in view of the fact that nearly all controllable, contributing conditions were maintained the same in the two series of studies.

In Table 19 are given the net-energy values of the mixed ration, of the alfalfa hay, and of the corn meal at four supermaintenance planes of nutrition, the values for the mixed ration being computed by a direct, difference procedure, and for the individual feeds by the net-proportional and the heat-increment-proportional methods.

The heat-increment and the net-energy values of the mixed ration differ considerably among themselves at the several planes of nutrition, and show by their divergence from the results of the year before that the experimental conditions in the two years' work must have differed in some important regards. These may have been the length and the arrangement of the experimental program and the extensive change in the live weight of the subjects, which have been discussed.

It is not easy to reconcile the net-energy values of the alfalfa hay and of the corn, as derived by the heat-increment-proportional method, with those obtained for the same feeds, by the same method, in the previous study (4). Since the values of the mixed ration determined at two planes of production in the earlier study were virtually identical, and therefore satisfied practical interests, it has been natural to accept them as essentially correct; but if they are correct then the values determined in the present study are distinctly unsatisfactory, and the derived net-energy values of the individual feeds, when computed by the heat-increment-proportional method, are of such obviously erroneous character as to serve only to discredit the means by which they were derived.

During the progress of these experiments the general ensemble of attendant conditions (except in the fasting periods) was so unusually favorable that the work appeared to be virtually perfect, but the results, calculated by the heat-increment-proportional method, as applying to the individual feeds, are impossible.

Aside from the question of validity, the failure of these net-energy values to agree with the parallel data from the earlier study was probably due to the various complications and difficulties in the fundamental nutritional situation and in the necessary experimental procedure, which have been enumerated in the foregoing discussion; but especially to the fact (previously stated) that in using the heat-incre-

ment-proportional method any error in the determination of the heat increments for maintenance—as contributed, for instance, by the value employed for the heat production of fast—is multiplied in computing these maintenance increments to increments for production. Also there was the element of error (see p. 64) originating in the unusual length of the experimental program and due directly to the lack of an accurate method for computing the heat production from one live-weight basis to another.

This series of experiments, which was devised primarily to reveal the relation of the heat production to the plane of nutrition, and not for the purpose of net-energy determinations, seems not to have been well adapted to serve the latter purpose.

The net-energy values of the hay and of the grain as computed by the net-proportional method were more consistent than those which have been discussed. The values of both grain and hay at the plane of one and one-half times maintenance are appreciably higher than the values representing the higher planes; but the latter values for the hay and for the grain agree rather well.

TABLE 22.—Average net-energy values of alfalfa hay, corn meal, and a mixed ration; obtained with four steers; the values of the individual feeds for body increase calculated by the net-proportional method

Experiment and steer No.	Values when used for maintenance (net energy per kilogram of dry matter)			Values when used for body increase (net energy per kilogram of dry matter)		
	Alfalfa hay and corn meal 1:1	Alfalfa hay	Corn meal	Alfalfa hay and corn meal 1:1	Alfalfa hay	Corn meal
238-47.....	Calories 2,241	Calories 1,385	Calories 3,111	Calories 1,557	Calories 963	Calories 2,162
238-36.....	2,218	1,395	3,050	1,541	969	2,125
240-60.....	2,257	1,419	3,102	1,482	932	2,037
240-57.....	2,426	1,562	3,293	1,610	1,037	2,185

In Table 22 are assembled the net-energy values of alfalfa hay, of corn meal, and of the mixed ration composed of these feeds from the present and the earlier experiment on the same subject. The values of the individual feeds for maintenance are computed by a direct, feed-difference procedure; and the values of the same at the several planes of nutrition above maintenance are computed by the net-proportional method and then averaged. These results seem promising, but they are few in number and their significance is not so thoroughly established as to warrant extended consideration.

In the previous study (4) the heat-increment-proportional method (4, p. 294) was preferred to the net-proportional method (4, p. 293) for computing net-energy values of single feeds for body increase, especially for the reason that in the use of the latter method the quantitative relation found to exist between the heat increments of a roughage and a concentrate for maintenance was reversed when this relation was computed as applying to feeding for body increase.

Such a reversal of relation of heat increments seemed inconsistent. Accordingly the heat-increment-proportional method was preferred, though the results obtained were recognized as peculiar and doubt was expressed as to the validity of the procedure. A somewhat different light is now thrown upon these two methods by a reexamination of the earlier work and by the results of the present study.

In comparing these two procedures, in the previous investigation (4), the heat increments were computed, as usual, on the basis of the dry matter of the feeds. It is now found, however (see the last columns of Tables 21 and 22 in the earlier paper and the last columns of Tables 18 and 19 of the present paper), that if the heat increments in either paper are compared on the basis of the metabolizable energy there is no such reversal as was observed of the quantitative relation of the heat increments of grain and hay at the different planes of nutrition.

In the present study there was an unusually good experimental basis for computing the heat-increment value of corn meal for body increase by the feed-and-plane-difference procedure, without the employment of either of the proportional methods. By comparing the heat production from a maintenance ration of alfalfa hay (period 13) with the heat production from a mixed ration consisting of about this same quantity of alfalfa hay plus an equal quantity of corn meal (period 9), it was found that the heat-increment value of the corn meal (1,280 Calories) was higher than that of the mixed ration of corn meal and alfalfa (1,127 Calories)—derived from a comparison

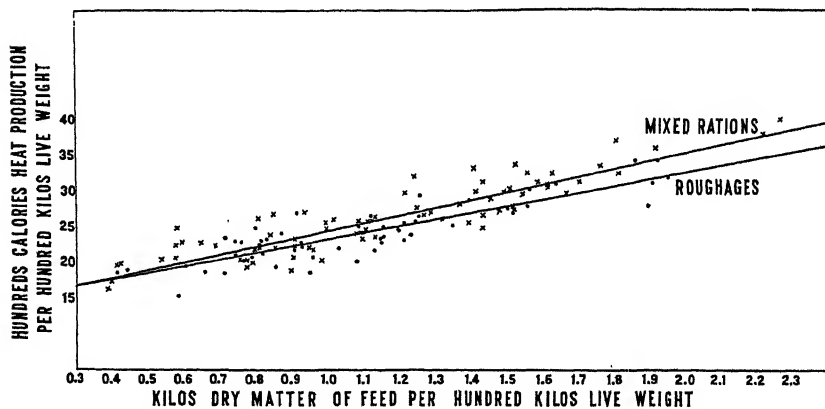


FIGURE 3.—The heat production of cattle as related to the dry matter of mixed rations and rations of roughage only

of the above-mentioned ration of corn and alfalfa (period 9) with another ration of the same composition fed at the maintenance level (period 1)—thus indicating that the heat-increment value of corn for body increase is actually higher than that of alfalfa hay—in harmony with the results of the earlier study (4) and of the present one as well, when computed by the net-proportional method.

In consideration of the fact that corn meal is considerably richer in metabolizable energy than is alfalfa hay it might be expected to have a higher heat-increment value if the relation were a direct and simple one. In this light the observations just cited seem consistent; but in the present as well as in the previous study the maintenance heat-increment value of corn meal is less than that of alfalfa hay—which directs attention to the fact, explained in the previous paper (4, p. 254), that the heat increment is a value of exceedingly complex origin; and in consideration of the number and diversity of its components, the above suggestion that these components, and the heat increments as a whole, do not vary, as affected by the plane of nutrition, in the simplest proportional manner, is not surprising.

Further light is thrown upon this matter by a statistical analysis, shown in Figure 3, of the results of published work of this institute, comparing the heat production from rations of roughage alone with that from mixed rations of grain and roughage.

Fifty-four experiments with roughage alone and 77 with grain and roughage are represented as two groups, by lines of regression representing the following equations for the relation between heat production and dry matter: For roughage rations $y = 0.931x + 1,386$; for mixed rations $y = 1.060x + 1,385$.

In viewing this graph the reader should bear in mind that the divergence of the line representing the mixed rations from that representing the roughage rations expresses only the influence of the grain in the mixed rations, and that a line representing grain alone would be much more divergent from that representing roughage. The graph shows that, on an average, the concentrates used in the mixed rations led to greater heat production than did the roughages—which tends to corroborate the net-proportional method, and suggests, therefore, that the assumption on which the heat-increment-proportional method is based, which assigns lower heat-increment values to the corn meal than to the alfalfa hay (Table 19), is incorrect.

Thus, the average heat-increment value per kilogram of dry matter of the mixed rations represented in this graph was 1,060 Calories, and of the rations of roughage alone, 931 Calories, and in the present study almost identical values were derived; that is, 1,076 Calories for the mixed ration and 930 Calories for the alfalfa hay, for body increase, when computed by the net-proportional method; while the heat-increment-proportional method assigned to alfalfa hay an average heat-increment value of 1,527 Calories. The heat-increment value and the corresponding net-energy value of the corn meal, as obtained by the direct comparison of data from periods 9 and 13 (p. 72), agree fairly well with the corresponding values computed by the net-proportional method. These values for heat increment were 1,280 Calories and 1,312 Calories, and for net energy, 1,835 Calories and 1,911 Calories, respectively, as computed by the two methods.

Also, the net-energy values obtained by the net-proportional method are in fair agreement with the values derived for the same feeds by Armsby (1, p. 660) and by two of the present writers (3, p. 1098).

In Table 23 are assembled heat-increment values of alfalfa hay determined directly, in supermaintenance periods in which the hay was fed by itself (experiments 212 and 216), for comparison with the heat-increment values of alfalfa hay, from the last two years' work, computed by the net-proportional and by the heat-increment-proportional methods.

The results computed by the net-proportional method agree fairly well with the directly determined values. The results computed by the heat-increment proportional method are much higher and more variable—higher, as a result of the difference in the assumptions on which the methods are based, and more variable because of the multiplication of experimental errors by the heat-increment-proportional computation.

TABLE 23.—Comparison of the heat-increment values of alfalfa hay (1) determined directly and computed (2) by the net-proportional and (3) by the heat-increment-proportional methods

Experiment No.	Periods compared	Heat-increment values determined—		
		Directly	By the net-proportional method	By the heat-increment-proportional method
		Calories	Calories	Calories
212 (3)	3 and 1.....	980		
216 (3)	4 and 2.....	934		
	6 and 5.....	1,056		
	Average.....	990		
238 (4)	1 and 7.....		957	1,286
	2 and 8.....		984	1,342
	3 and 7.....		1,043	1,425
	4 and 8.....		1,036	1,425
	1 and 3.....		830	1,145
	2 and 4.....		802	1,420
	1 and 5.....		975	1,436
	2 and 6.....		991	1,034
	1 and 7.....		1,016	1,520
	2 and 8.....		952	1,841
	1 and 9.....		945	1,393
	Average.....		957	1,470
240				

* Results presented in present paper.

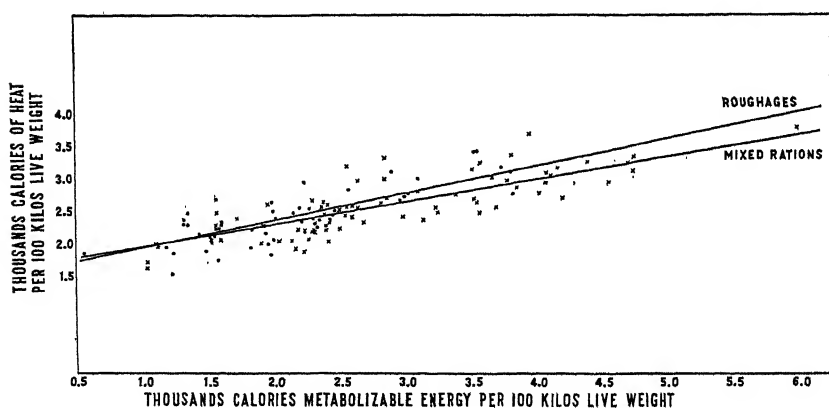


FIGURE 4.—The heat production of cattle as related to the metabolizable energy of mixed rations and rations of roughages only

In relation to the relative energy expenditure of utilization of grain and of roughage feeds, in general, it is significant (1) that the quantitative relationship of heat-increment values of grain and roughage shown in Figure 3 would be reversed if the heat production were related to the metabolizable energy instead of to the dry matter, as in Figure 4; (2) that the higher rate of increase in heat production from grains than from roughages—with increase in dry matter of feed consumed—is due in part to the higher metabolizable energy content of the grains (fig. 3); and (3) that the higher rate of increase in heat production per unit of metabolizable energy of roughage is presumably due in part each to the greater quantity of dry matter per unit of metabolizable energy, and to the more resistant physical character of the dry matter of such feeds. (Fig. 4.)

The following are the equations for the relation between the heat production and the metabolizable energy represented by the lines of regression of Figure 4: For roughage rations $y = 0.419 x + 1,526$; for mixed rations $y = 0.343 x + 1,615$.

It is the opinion of the writers that the evidence at hand does not afford a satisfactory basis for final choice between the foregoing methods of computation of the results of a net-energy determination; and they defer judgment on this matter until a considerable body of results shall have been obtained in experiments especially planned for the derivation of net-energy values, in the light of the present ideas of the authors as to essential requirements.

From the foregoing recountal of the difficulties and compromising conditions inherent in the determination of net-energy values it will be understood that these measures can not be considered as of absolute value; in fact the determination of net-energy values must be so rigidly and arbitrarily standardized and controlled that the results, in spite of the particularity of the method of determination, must be regarded as, to a considerable extent, conventional.

The net energy conception, however, expresses a simple, logical, and scientific point of view from which to regard the supremely important subject of energy metabolism; and a natural approach to the subject is through a study of the factors which affect the net energy. Also, no other measure of the energy values of foods has been proposed which has a scientific validity at all approaching that of net-energy, in spite of all the compromises involved.

An important problem in connection with the use of net-energy values is that of the terms in which they are to be stated; and the utmost practicable simplicity in the system is imperative.

Armsby, Kellner, and Møllgaard have all stated nutritive values based on the net-energy conception in terms of a single unit; that is, all three have stated the energy requirements for maintenance, for body increase, and for milk production in terms of values for body increase, though Armsby's production values were, by the method of determination, somewhat mixed with net energy for maintenance.

A somewhat different light is now thrown upon this problem—in the authors' minds, at least—by their studies on the heat production of fast; by their adoption of the directly determined heat production of fast as the base value in energy metabolism of cattle and as the measure of the net-energy requirement of maintenance; by their demonstration of the relation of the heat production to the plane of nutrition; and by the demonstration, by Møllgaard and themselves, of the essentially different net-energy value of a food for body increase, on the one hand, as compared with maintenance and milk production on the other.

Though a simple system of designation of nutritive values seems no less desirable, on account of this recent progress, it is now clear that to use a single net-energy value, as a measure of nutriment for maintenance, milk production, and body increase, would be to apply it to a much more complicated situation, as to economy of utilization of food energy, than was formerly understood.

The question above suggested is, fundamentally, whether the essential difference in the economy of utilization of food energy for maintenance and milk production, as compared with body increase, shall be expressed in terms of different net-energy values, or whether,

for the sake of convenience, one net-energy value is to be used, and the difference in economy of utilization of food energy is to be expressed in the feeding standard. The authors are inclined to reserve their decision on this point, pending the settlement of the more urgent, fundamental problems involved in the net-energy determination.

In the light of the foregoing discussion, therefore, it is proposed to adopt a standard arrangement of the experimental periods in a program of determinations of these measures. Thus, in order to derive the greatest profit from such experiments, with cattle, it is advantageous to determine the net-energy values of a grain and of a roughage, for maintenance and for body increase, in one experimental program; and the writers suggest that the schedule of treatments should be arranged as follows:

Treatment	Period
Roughage and grain; two times maintenance-----	1
Roughage and grain; maintenance-----	2
Roughage; maintenance-----	3
Fast-----	4
Slaughter; to determine surface area-----	5

This arrangement provides, as a standard procedure, that fast shall follow maintenance; and also that there shall be the minimum differences between the live weights of the animal in the periods which must be compared, thus requiring the minimum correction of the heat production, as observed, in computing to the basis of one live weight.

The above schedule of experimentation, however, is not suggested as one to be adhered to in an absolutely fixed manner. For special purposes it may be varied; but it seems to be well adapted, as it stands, to serve the most extensive of present requirements.

In the derivation of such a system of measures it is essential that the energy metabolism be referred to a definite base value; and a series of experiments has been conducted at this institute having for their object the definition—as such a base value, and as the measure of the maintenance requirement of net energy—of the conditions of determination of the heat production of fast.

Though this study is not yet finished the authors consider it desirable to make as complete a statement as possible at this time of their position with reference to the problem of determination of net-energy values. They therefore anticipate the full publication of this research by proposing the following statement of standard conditions for the determination of the heat production of fast, as the base value in energy metabolism, and as the measure of the maintenance quota of net energy: The heat production of the first day, beginning either in the morning or the evening, following the appearance, within the animal, of a status of metabolism characterized by the nonprotein respiratory quotient of fat—this measurement to follow a preparatory feeding at the plane of energy equilibrium.

SUMMARY

A series of 15 experiments was conducted, with 2-year-old Short-horn steers as subjects, by direct heat measurements checked by indirect calorimetry, for the study of energy metabolism in relation to the plane of nutrition, especially as bearing on the problem of method of determination of net-energy values of feeding stuffs.

The energy metabolism was studied at seven planes of nutrition, namely, (1) fast, (2) half of the maintenance requirement, (3) maintenance, (4) half more than maintenance, (5) two times maintenance, (6) two and a half times maintenance, and (7) three times maintenance.

In the feeding periods the rations were composed of corn meal and alfalfa hay, in equal weights of dry substance, except in one period with each steer, in which the ration was alfalfa hay alone.

In the preparation of the steers for the fasting heat measurement grain alone was given during the last seven days' feeding. This treatment, together with the physicking of the steers, reduced the contents of the alimentary tract to negligible quantities.

With the heat production of the fourth day of fast as the base value, the heat production increased slowly between fast and maintenance, and much more rapidly above maintenance, but with a decreased rate of rise between the planes of twice and three times maintenance.

The curve of heat production in relation to the plane of nutrition was found, therefore, to be a reversed or S curve.

The heat production of fast being considered as including two factors, a waste heat of utilization of body nutrients katabolized, and a theoretical minimum base value, including no such waste—the curvature of the line of heat production in relation to increasing food consumption is interpreted as resulting from (1) the increasing concentration of metabolites circulating in the blood; (2) the change in the proportions of protein, fat, and carbohydrate katabolized, with increase in the katabolism of food nutrients and decrease in the katabolism of body nutrients; (3) the energy expense of synthesis of body nutrients (fat from carbohydrates); and (4) the decreased metabolizability of the food at the higher planes of nutrition.

The heat increments between the lower planes of nutrition were considered to be less than the true energy expense of food utilization by the amount of the waste heat of utilization of body nutrients katabolized.

Attention is called to the fact that on account of the extensive fermentation of carbohydrates, with accompanying heat production, in the ruminant alimentary tract, the relative energy expenses of utilization of protein, fat, and carbohydrates, as found in other animals, do not prevail in ruminants.

The authors conclude that the most practicable possibility of deriving a system of energy values of feeding stuffs, to serve as guides in practice, requires the continued adherence to the general point of view and method of Armsby, but modified, in the light of recent findings at this institute; (1) by the adoption of the standardized, directly observed heat production of fast as the base value in the energy metabolism of cattle, and as the measure of the maintenance requirement of net energy, and (2) by the recognition of the facts as to the relation of the energy metabolism to the plane of nutrition, which imply the existence of a fundamentally different net-energy value of a feeding stuff at each point of observation, in relation to the plane of nutrition, and which necessitate a practical recognition of the different rate of economy of utilization of food energy for body increase as compared with maintenance.

The authors suggest the following statement of standard conditions for the determination of the heat production of fasting cattle, as the

base value in energy metabolism, and as the measure of the maintenance quota of net energy: The heat production of the first day—beginning either in the morning or the evening—following the appearance, within the animal, of a status of metabolism characterized by the nonprotein respiratory quotient of fat; this measurement to follow a preparatory feeding at the plane of energy equilibrium.

The most difficult unsolved problem in net-energy determination is the separation of the net energy of grain from that of roughage (hay), since neither can be fed alone, at practical levels of production, and since combining the two has the effect of altering the rates of economy at which they are utilized.

Methods of computation of the results of net-energy determinations are critically discussed, and a standard schedule of experimentation for net-energy determinations is proposed.

LITERATURE CITED

- (1) ARMSBY, H. P.
1917. *THE NUTRITION OF FARM ANIMALS*. 743 p., illus. New York.
- (2) FORBES, E. B.
1905. GRAIN RATIONS FOR DRY LOT HOG FEEDING. *Missouri Agr. Expt. Sta. Bul.* 65, p. 27-92.
- (3) ——— and KRISS, M.
1925. REVISED NET-ENERGY VALUES OF FEEDING STUFFS FOR CATTLE. *Jour. Agr. Research* 31: 1083-1099.
- (4) ——— BRAMAN, W. W., and KRISS, M., with the collaboration of JEFFRIES, C. D., SWIFT, R. W., FRENCH, R. B., and others.
1928. ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION. *Jour. Agr. Research* 37: 253-300, illus.
- (5) HOGAN, A. G., and SKOUBY, C. I.
1923. DETERMINATION OF THE SURFACE AREA OF CATTLE AND SWINE. *Jour. Agr. Research* 25: 419-430, illus.
- (6) MOULTON, C. R.
1916. UNITS OF REFERENCE FOR BASAL METABOLISM AND THEIR INTER-RELATIONS. *Jour. Biol. Chem.* 24: 299-320, illus.

BIOLOGICAL VALUES AND SUPPLEMENTARY RELATIONS OF THE PROTEINS IN ALFALFA HAY AND IN CORN AND SUNFLOWER SILAGE¹

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INTRODUCTION

The conception of the net-energy values of feeds, as worked out by Armsby (1),³ renders accurate comparisons of feeds possible in respect to their energy content for various types of production and maintenance. Owing to the complexity of the protein molecule, in which quantitative and especially qualitative differences occur according to the source, no such direct method of comparison has been developed for the protein content. A molecule of a complete protein may consist of a chain of 30 (14) or more alpha amino acids, some of which are complex and others simple, some present in abundance, others only as traces, according to the nature of the protein.

The most accurate method of evaluating proteins available at the present time appears to be that employed by Thomas as modified by Mitchell (6). This method takes into consideration the body nitrogen in both the urine and feces during protein feeding as determined by feeding rations nearly free of nitrogen.⁴ This method can be used with a single protein, as well as with protein mixtures. It is particularly valuable for determining in which feed combinations the protein mixtures are most effective in supporting production and maintenance.

It is a well-established fact that proteins deficient or low in one or more of the necessary amino acids can be rendered highly efficient through the proper combination with proteins rich in the missing acids.

PLAN OF THE EXPERIMENTS

The chief aim in this study was to determine the biological values of the proteins in alfalfa hay, sunflower silage, and corn silage when fed singly and in combination to lambs. Mixtures of 3 parts of silage to 1 of hay were selected, since these are so commonly used in practice.

In studying any feed or feed combination, six lambs were fed for a 10-day preliminary and a 10-day experimental period. Nearly nitrogen-free rations were fed before and at the conclusion of the experimental series of rations. The order of feeding as actually planned and carried out was as follows: (1) Nearly nitrogen-free rations; (2) alfalfa hay; (3) sunflower silage; (4) 1 part alfalfa hay, 3 parts sunflower silage, by weight; (5) corn silage; (6) 1 part

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² Thanks are due the members of the Division of Chemistry, in whose laboratory the writer did the analytical work, and to H. Hackedorf, head of the Department of Animal Husbandry, for helpful suggestions.

³ Reference is made by number (italic) to "Literature cited," p. 96.

⁴ Protein in this discussion is considered to be nitrogen $\times 6.25$.

alfalfa hay, 3 parts corn silage by weight; (7) nearly nitrogen-free rations.

The entire field work was completed in 140 days, during which a quantitative collection of feces and urine was made. However, between each 10-day period, at least 1 or 2 days were necessary for a complete cleaning of the crates before a new period was started.

There were three metabolism crates available for use, and while three lambs were being fed, the others were undergoing preliminary feeding, preparatory to the next period. In this manner the field and chemical work could go on simultaneously with little danger of losing samples through spoilage or deterioration.

CHEMICAL DETERMINATIONS

All nitrogen determinations were made by the official Kjeldahl-Gunning-Arnold method for organic nitrogen. Official methods of analysis were used throughout this study.

FACTORS WHICH AFFECT BIOLOGICAL VALUES

The nutritive value of the proteins in any single feed mixture is taken as the total nitrogen retained by the experimental animal in percentage of the total nitrogen absorbed. This percentage is called the biological value of the protein and measures the value of a protein in all the anabolic reactions of the body.

It is apparent that differences in digestibility are not involved in determining the biological value of proteins. However, the biological value of the protein is expected to vary (?) with the use to which the protein is put by the body. These differences are related to the amino acid make-up of the protein as compared with the several separate requirements for amino acids. Furthermore, under any one set of experimental conditions the biological value of a protein is fixed by its constitution.

The biological value is, then, (5) determined by the ratio of the percentages in food protein and body complex, respectively, of the amino acid for which this ratio has the lowest value, unless the body has the capacity to synthesize that particular amino acid from others.

Two assumptions in such studies are ordinarily made: (1) That the undigested fraction of a protein is of the same amino acid make-up as that digested; (2) that in protein hydrolysis, amino acids split off from the molecule at equal rates, or else that tissues can store those splitting off first until others form, so that optimum proportions of amino acids are secured for body needs.

It is also assumed that no protein is catabolized for energy purposes until all requirements for body proteins are met, excluding, of course, that protein or its fraction not available for body needs.

Mitchell (?) has demonstrated for several proteins that an increase in the level of protein intake results in a lowering of the biological value of the protein. The lowering occurs as the concentration of the protein in the ration increases, but is not proportional to the quantity of protein intake, as influenced by variations in feed consumption.

During the feeding of nearly nitrogen-free rations for ideal results, no protein of the body should be used as a source of energy, except

those fractions of the protein molecule^r not completely utilized for maintenance and various forms of production.

The body's contribution of nitrogen to the urinary and fecal excretions during periods of protein feeding then is measured by the nitrogen content of the urine and feces of the experimental animal when receiving a near-nitrogen-free ration rich in net energy, the feed intake being such as to exceed liberally the energy maintenance requirement.

If the fecal nitrogen excretion on a nitrogen-free diet is to be taken as a measure of the excretion of metabolic fecal nitrogen during subsequent experimental periods in which rations containing protein are fed, it is advisable to equalize the fiber content as nearly as possible in both the nonnitrogenous and nitrogenous rations. Mitchell (6) points out that failure to equalize the fiber results in an underestimation of metabolic nitrogen and to an overestimation both of food nitrogen retained in the body and of the absorbed nitrogen, the numerator and denominator, respectively, of the fraction determining the biological value. For this reason this error is not considered very serious.

It has also been shown that the excretion of nitrogen in the feces of animals on diets containing minimal amounts of nitrogen (0.04 to 0.08 per cent) varies directly with the amount of food consumed.

The best method, then, of estimating metabolic nitrogen in the feces of animals on rations containing protein is to determine in a period of nonnitrogenous feeding the excretion of fecal nitrogen per gram of dry matter of the food consumed and to apply this figure to the amount of dry matter of the protein-containing food consumed in subsequent experimental periods.

An indirect way of measuring urinary nitrogen of endogenous origin is to eliminate entirely the exogenous catabolism by the feeding of a nitrogen-free diet, and inducing the animal to eat enough of the ration to provide for its energy requirements, thus preventing oxidation of body tissue with its accompanying loss of nitrogen, which can not properly be called endogenous nitrogen. The assumption is made that the catabolism of body substances containing nitrogen, occurring during feeding of nitrogen-free rations high in energy, continues at the at the same level during protein feeding.

The quantity of nitrogen in the urine per 100 gm. of body weight during periods of feeding nearly nitrogen-free rations, when applied to the weights of the same animals fed protein rations, is then the measure of the endogenous urinary nitrogen.

The nitrogen in the urine and feces does not completely account for the total loss of nitrogen from the body. To these should be added the loss through wool and epidermis. In sheep, however, this quantity is small, and is disregarded, especially in short-time experiments.

The biological value expressed in a mathematical form is as follows:

Biological value =

$$100 \frac{[(\text{Food N} - \text{Food N in feces}) - (\text{Urine N} - \text{Body N in urine})]}{\text{Food N} - (\text{N in feces} - \text{body N in feces})}$$

or

$$\text{Biological value} = 100 \frac{\text{Food N retained}}{\text{Absorbed N}}$$

Mitchell (9) has pointed out that the determination of the biological value is unaffected by the position of the experimental period in rela-

tion to the near-nitrogen period. Among 17 possible comparisons, 9 favored the view that low-nitrogen feeding increases the biological value in an immediately subsequent period, 6 were opposed to this view, and 2 comparisons showed no difference.

NITROGEN METABOLISM STUDIES

The lambs were fed in metabolism crates previously described (13). Every feed combination was studied with six lambs, each fed over a 10-day preliminary and a 10-day experimental period.

The urine samples were collected daily, and the largest portion of feces was also collected daily except at the end of each period, when the crates were cleaned and washed. The urine was kept in carboys and the feces in air-tight galvanized iron cans. The total urine and feces from each sheep were carefully sampled (2) and analyzed soon after sampling. All the sampling was done rapidly and at fairly low temperature, and the samples were immediately analyzed to minimize losses in moisture.

Body weights were taken at the beginning and at the end of each period.

The roughage feed for any particular trial was weighed out at one time and placed in paper sacks. A representative sample taken at this time for chemical analysis served as the basis for computing the nutrients fed.

Fresh silage was taken from the silo each morning, completely mixed, and the daily allowance for each lamb weighed out and placed in air-tight jars. Occasional handfuls of silage were placed in a sample jar for analysis. These daily samples were dried, and at the end of each 10-day experimental period a composite sample was prepared for chemical study.

Unconsumed feed was collected daily, air-dried, ground, and sampled. The nutrient intake was then computed by subtracting the nutrients in the refuse from those in the feed consumed. In one instance during the feeding of the near-nitrogen-free rations the total grams of nitrogen in the unconsumed feed exceeded the nitrogen in the feed consumed. A careful recheck led to the possible explanation that the saliva in the feed refused contributed the excess nitrogen.

A further description of methods is given in a previous paper (13)

NEARLY NITROGEN-FREE RATIONS AS A MEANS OF DETERMINING PROTEIN MAINTENANCE REQUIREMENTS

The fact is well established that when a ration containing net energy in excess of the maintenance requirement is fed the total nitrogen excreted in the urine and feces is less than during complete fasting. The feeding of nonnitrogenous nutrients tends to diminish the catabolism of proteins to the minimum. However, even a liberal supply of energy will not completely prevent the breaking down of tissues.

Robison (12) assumed that the minimum nitrogen requirement of maintenance is represented by the sum of the nitrogen in the urine and that in the feces during periods of feeding liberal nearly nitrogen-free rations.

Mitchell (8), however, points out that the maintenance requirement for nitrogen by any animal may be measured by the total daily excretion of nitrogen in the urine alone, to the exclusion of the fecal nitrogen,

Although the fecal nitrogen is of body origin, it varies with the dry matter consumed, and would vary with the type of maintenance ration used, increasing with a corresponding increase of the ration. It appears that the fecal nitrogen should be considered as a wastage to be subtracted from the feed nitrogen.

Table 1 contains the data of twelve 10-day metabolism experiments, during which nearly nitrogen-free rations consisting of linseed oil, corn-starch, cane sugar, and selected stems of Albit wheat straw were fed. The average of 0.664 pound of protein per 1,000 pounds live weight for lambs averaging 24.34 kgm., determined according to Robison (12), compares favorably with the requirements of Schulze and Märcker cited by Armsby (1). These investigators recommend 0.653 pound digestible crude protein daily per 1,000 pounds live weight for maintenance.

A study of the individual maintenance requirements shows a minimum of 0.495 and a maximum of 0.886 pound of digestible crude protein required daily for maintenance per 1,000 pounds live weight.

Data for the urinary nitrogen are also reported in Table 1, and from these figures the maintenance requirements may be computed according to the suggestion of Mitchell (8).

TABLE 1.—Maintenance requirements of lambs averaging 24.34 kilograms in weight as determined by 10-day metabolism experiments during which nearly nitrogen-free rations were fed

[Totals are for 10-day experimental period]

Trial No.	Lamb No. ^a	Average weight	Nitrogen in feed consumed	Fiber in feed fed	Total urinary nitrogen	Total fecal nitrogen	Total of urinary and fecal nitrogen	Protein ^b per 1,000 pounds live weight
		Kilograms	Per cent	Per cent	Grams	Grams	Grams	Pounds
5.....	1 e.....	28.24	None.	15.99	12.38	12.85	25.23	5.62
	3 w.....	30.73	0.11	15.99	9.58	19.57	29.15	5.93
	5 e.....	20.07	.11	15.99	7.78	12.29	20.07	6.25
6.....	2 e.....	22.34	.10	15.99	5.90	12.14	18.04	5.05
	4 w.....	29.37	.07	15.99	8.61	17.62	26.23	5.58
	6 w.....	22.34	.12	15.99	4.33	13.38	17.71	4.95
15.....	1 e.....	25.17	.11	13.30	10.54	18.30	28.84	7.16
	3 w.....	28.58	.12	13.10	8.58	26.29	34.87	7.62
	5 e.....	19.28	.10	12.83	6.00	20.15	26.15	8.48
16.....	2 e.....	20.07	.11	15.07	4.15	20.34	24.49	7.62
	4 w.....	26.20	.14	15.07	9.58	18.00	27.58	6.58
	6 w.....	19.73	.13	15.07	9.45	18.52	27.97	8.86
Average.....		24.34	.11	15.03	8.07	17.45	25.53	6.64
Average per day.....					.807	1.745	2.553	.664

^a Letters e and w indicate ewe and wether respectively wherever used after sheep number in all tables.

^b To be supplied in net-protein form.

Robison (12) points out that starch prepared in the commercial way contains some nitrogen and that its value to the body is not known. If the value of this nitrogen in the nearly nitrogen-free diet is zero, then the real nitrogen requirements will be less than that computed from the output by the full amount of such a nitrogen intake, since the latter must be excreted in addition to the nitrogen that results from the catabolism of body protein. If, on the other hand, the value of this nitrogen for replacement or increase of body nitrogen is 100 per cent, then it will spare or produce an equal amount of body nitrogen, and the observed output during feeding of the nitrogen-free ration will represent the actual minimum requirement. If, however, the value of

this nitrogen for replacement or increase of body nitrogen is 75 per cent, then the other 25 per cent appears in the excreta, and the excretory nitrogen should be reduced by this amount.

The average dry-matter intake per lamb during the 12 nearly nitrogen-free ration periods was 273.8 gm. daily. With an 0.11 per cent average nitrogen content, this means an average daily intake of 0.301 gm. of nitrogen. If this nitrogen has a value of zero, then this quantity should be subtracted from the 2.554 gm., which is the average total nitrogen excretion from both the feces and urine. If this is done the maintenance requirement of the lambs is lowered by almost 12 per cent.

The data in Table 1, in addition to the dry-matter intake, were used in computing the fecal nitrogen per gram of dry matter ingested and the urinary nitrogen per 100 gm. of body weight per day. Tables 2 and 3 contain these data arranged according to trial and according to lamb, with necessary averages which are used in computing the biological values of the proteins in rations fed during intervening periods.

TABLE 2.—*Fecal nitrogen per gram of dry matter ingested and urinary nitrogen per 100 grams body weight, based on trials during which nearly nitrogen-free rations were fed*

[Totals are for 10-day experimental periods]

Trial No.	Lamb No.	Average weight	Dry matter ingested	Total fecal nitrogen	Total urinary nitrogen	Fecal nitrogen per gram dry matter ingested	Urinary nitrogen per 100 grams body weight per day
		<i>Kilograms</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Gram</i>	<i>Gram</i>
5.....	1 e.....	28.24	1,502	12.85	12.38	0.0086	0.0438
	3 w.....	30.73	4,155	19.57	9.58	.0047	.0312
	5 e.....	20.07	2,176	12.29	7.78	.0057	.0388
6.....	2 e.....	22.34	1,868	12.14	5.90	.0065	.0264
	4 w.....	29.37	2,908	17.02	8.61	.0061	.0293
	6 w.....	22.34	2,341	13.38	4.33	.0057	.0194
15.....	1 e.....	25.17	2,924	18.30	10.54	.0063	.0419
	3 w.....	28.58	3,736	26.29	8.58	.0070	.0300
	5 e.....	19.28	2,441	20.15	6.00	.0083	.0311
16.....	2 e.....	20.07	2,862	20.34	4.15	.0071	.0207
	4 w.....	26.20	2,722	18.00	9.58	.0066	.0360
	6 w.....	19.73	3,218	18.52	9.46	.0058	.0470

TABLE 3.—*Fecal nitrogen per gram dry matter ingested and urinary nitrogen per 100 grams body weight, based on trials during which nearly nitrogen-free rations were fed*

Lamb No.	Trial No.	Fecal nitrogen per gram dry matter	Urinary nitrogen per 100 grams body weight	Average fecal nitrogen per gram dry matter	Average urinary nitrogen per 100 grams body weight
		<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>
1.....	5.....	0.0086	0.0438		
	15.....	.0063	.0419		
	6.....	.0065	.0264	0.0075	0.0428
2.....	16.....	.0071	.0207		
	5.....	.0047	.0312	.0068	.0236
3.....	15.....	.0070	.0300		
	6.....	.0061	.0293	.0059	.0306
4.....	16.....	.0066	.0366		
	5.....	.0057	.0388	.0064	.0330
5.....	15.....	.0083	.0311		
	6.....	.0057	.0194	.0070	.0350
6.....	16.....	.0058	.0470		
				.0058	.0337

The make-up of the nearly nitrogen-free rations is reported in Table 1 and 5. Their content of the various feeds on a percentage basis is as follows:

Feeds:	Trial Nos. 5 and 6 (per cent)	Trial Nos. 15 and 16 (per cent)
Straw ⁵ -----	51.5	47.1
Starch ⁶ -----	31.0	25.7
Sugar-----	16.0	25.7
Oil-----	1.5	1.5
Total-----	100.0	100.0

During trials 15 and 16 the percentage of sugar was slightly increased and the starch and straw percentages increased. The changes resulted in a somewhat more palatable ration, and no nauseating effect accompanied the larger sugar intake.

At no time during the experimental periods were any digestive disturbances noticed, the lambs appearing normal in every way. The feces were extremely light in color, as might be expected from the make-up of the ration, but they were of normal consistency.

The lambs had free access to salt during all preliminary periods, and salt was daily fed by hand during experimental periods in quantities of one-fourth to one-third of an ounce according to the quantity (4) recommended by Kellner.

Precaution against eating of bedding straw during preliminary periods of feeding nitrogen-free rations seemed unnecessary since the straw used for bedding was the same kind as that used in the daily ration. During other periods the bedding was placed in burlap sacks.

The lambs were fed in separate pens, one lamb to a pen, during all preliminary periods, and were kept singly in the metabolism crates. Since all fleeces were carefully trimmed, including the dung tags, there was very little wool mixed with the feces during collection periods. Furthermore, close observation failed to show that the lambs at any time were eating their own wool.

Results previously obtained by the writer in nitrogen metabolism studies with lambs, together with results reported by other investigators for man and several species of the lower animals, are shown in Table 4. The total urinary nitrogen of lambs, per kilogram of live weight per day (0.0331 kgm.) is strikingly similar to that of man. The urinary nitrogen of the albino rat and the dog are seven and five times greater, respectively, than that of the lamb.

⁵ Selected stems of ripe Albit wheat straw, very low in nitrogen, were used.

⁶ Buffalo starch is made by the wet process of grinding and separating starch from corn and is very low in nitrogen. The starch containing gluten is settled on long tables, separating the gluten. The starch is then flushed from the tables, washed on vacuum filters with fresh water to substantially remove soluble matter, dried in kilns, and ground.

TABLE 4.—A comparison of the urinary nitrogen excreted per kilogram of body weight on high energy nearly nitrogen-free rations for man and various species of animals ^a

Observer and date	Animal	Weight	Ration	Total nitrogen in urine per kilogram live weight
		<i>Kilograms</i>		<i>Gram</i>
Folin (1905).....	Man.....	85.7	Starch, cream, 1 gm. N.....	0.0420
Graham and Poulton (1912).....	do.....	62.4	Starch, cream, 0.912 gm. N.....	.0445
Do.....	do.....	72.4	Starch, cream, 1.23 gm. N.....	.0408
af. Klercker (1907).....	do.....	88.0	Low N.....	.0319
Robinson (1922).....	do.....	60.5	Carbohydrate, fat, 0.3 gm. N.....	.0352
Do.....	do.....	58.0	do.....	.0355
McCollum (1911).....	Pig.....	10.9	Carbohydrate.....	.0495
Do.....	do.....	68.4	do.....	.0387
Mendel and Rose (1911).....	Rabbit.....	1.74	do.....	.1260
Murlin (1907, 2).....	Dog.....	11.3	do.....	.1580
Mitchell (6) (1923).....	White rat.....	.134	N-free.....	.2198
Sotola (1928).....	Black-faced lamb.....	24.3	Nearly N-free.....	.0331

^a With the exception of the last two lines, this table is a part reproduction of Robinson's Table 1 (12, p. 115).

DISCUSSION OF BIOLOGICAL VALUES

A complete report of metabolism data, upon which the computation of the biological values is based, is given in Table 5. Nearly nitrogen-free rations fed during trials 5 and 6, and 15 and 16, the beginning and end of the series, form the basis for determining the body nitrogen in the urine and feces during the intervening periods. On the basis of these trials, the fecal nitrogen per gram of dry matter consumed and the urinary nitrogen per 100 gm. of body weight were determined for each lamb. These data were then applied to the corresponding lambs during the intermediate periods of protein feeding, in computing the body nitrogen in the urine and feces, in proportion to a varying body weight and dry-matter intake.

The data for the nearly nitrogen-free rations are contained in Table 2 and rearranged according to lamb numbers in Table 3.

The results with lamb 1 (Table 3) show that during trial 5 the fecal nitrogen per gram of dry matter consumed was 0.0086 gm. and during trial 15, 0.0063 gm. These results can be averaged and the average applied to the intervening periods, or else the decrease can be applied in a linear fashion to the intervening periods. Both methods were used, and in nearly all instances little difference in the biological values can be detected. For comparison, these values computed by applying the results of the nearly nitrogen-free rations in a linear fashion, or by averaging them, may be found in the last two columns of Table 5. The former method (6) is given preference.

TABLE 5.—Summary of metabolism experiments upon which the determination of the biological values of proteins in alfalfa hay, corn silage, sunflower silage, and these feeds fed in mixtures, is based

[Totals are for 10-day experimental periods]

Ration	Trial No.	Sheep No. ^a	Body weight			Feed intake, dry matter	Nitrogen intake	Fecal nitrogen	Body nitrogen in feces ^b	Food nitrogen in feces	Absorbed nitrogen	Total nitrogen in urine	Body nitrogen in urine	Food nitrogen in urine	Food nitrogen retained	Biological value ^c of protein by—
			Initial	Final	Average											Linear method
Linseed oil, sugar, starch, wheat straw.	5	1 e	Kilo-grams	Kilo-grams	Kilo-grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	
		3 w	27.90	28.58	28.24	1,502	None.	12.85	12.92	8.06	233.79	103.13	13.81	94.32	139.47	59
		5 e	31.52	29.94	30.73	4,155	4.88	19.57	19.52	20.12	202.78	105.22	9.85	95.37	107.41	52
Do.	6	4 w	20.64	19.50	20.07	2,176	2.41	12.29	12.40	6.40	166.23	81.10	8.26	72.84	93.39	56
		6 w	23.36	21.31	22.34	1,868	1.99	12.14	12.14	1.36	229.28	81.02	6.91	74.71	154.57	67
		4 w	31.07	27.67	29.37	2,908	2.11	17.62	17.73	4.45	238.82	138.73	11.26	127.47	101.35	44
Alfalfa hay.	11	1 e	22.68	21.99	22.34	2,341	3.07	13.38	13.34	4.16	170.79	80.27	9.09	71.18	99.61	58
		3 w	32.20	32.20	32.20	10,633	241.85	87.81	79.75	1.36	229.28	81.02	6.91	74.71	154.57	67
		5 e	31.30	33.11	32.20	9,953	222.90	77.85	52.51	4.45	238.82	138.73	11.26	127.47	101.35	44
Do.	12	4 w	23.13	24.04	23.59	7,502	172.63	58.91	55.59	4.16	170.79	80.27	9.09	71.18	99.61	58
		6 w	28.58	29.94	29.26	10,099	230.64	70.03	68.67	1.36	229.28	81.02	6.91	74.71	154.57	67
		4 w	33.79	34.47	34.13	10,411	233.27	70.04	63.59	4.45	238.82	138.73	11.26	127.47	101.35	44
Sunflower silage.	7	1 e	24.72	24.49	24.61	7,808	174.95	48.67	44.51	4.16	170.79	80.27	9.09	71.18	99.61	58
		3 w	27.22	25.86	26.54	3,644	40.87	30.05	29.88	1.36	229.28	81.02	6.91	74.71	154.57	67
		5 e	23.03	21.22	22.12	3,044	40.87	29.31	28.58	10.73	30.14	21.36	8.72	12.64	17.50	38
Do.	8	4 w	19.30	19.30	19.30	3,459	40.87	26.99	21.10	5.89	34.45	13.02	7.31	5.71	28.74	83
		6 w	22.68	23.13	22.91	3,522	39.94	24.56	22.25	1.31	38.63	14.56	5.84	8.79	29.01	77
		4 w	20.26	20.99	20.62	2,445	27.36	19.68	13.10	4.52	22.84	9.61	5.65	10.03	0.81	20
		6 w	21.55	21.77	21.66	3,706	40.05	26.26	21.12	5.14	34.91	13.58	5.22	10.36	24.55	70

^a e and w are used, respectively, to indicate ewe or wether.^b Whenever the figure for body nitrogen in the feces is larger than the figure for fecal nitrogen, the food nitrogen is assumed to be 100 per cent digestible.^c To compute the biological values of proteins, the body nitrogen in the feces and urine for any particular lamb during periods of protein feeding is computed from the fecal nitrogen per gram dry matter and from the average urinary nitrogen per 100 grams body weight as determined during periods of nearly nitrogen-free feeding for the same lamb. (5, 6, 15, and 16.) The biological values in the last column are based on the averages of the nearly nitrogen-free period. The values in the next to the last column are based on differences of values 5, 6, 15, and 16. These differences are applied in a linear fashion to the intervening trials. In all cases the results with a certain lamb are applied throughout to all experiments with that animal.

TABLE 5.—Summary of metabolism experiments upon which the determination of the biological values of proteins in alfalfa hay, corn silage, sunflower silage, and these feeds fed in mixtures, is based.—Continued

[Totals are for 10-day experimental periods]

Ration	Trial No.	Sheep No.	Body weight			Feed intake, dry matter	Nitrogen intake	Fecal nitrogen	Body nitrogen in feces	Food nitrogen in feces	Absorbed nitrogen	Total nitrogen in urine	Body nitrogen in urine	Food nitrogen in urine	Food nitrogen retained	Biological value of protein by—
			Initial	Final	Average											Linear of average method
1 part alfalfa hay, 3 parts sunflower silage.	9	1 e	Kilo-grams	27.90	27.67	5.944	103.30	41.17	46.36	7.64	103.30	55.63	12.00	43.63	59.67	57
		3 w	grams	29.26	29.48	5.968	103.06	40.63	32.99	7.64	96.02	51.83	9.05	42.78	53.24	55
Do.	10	5 e	grams	21.55	21.55	5.670	101.40	33.11	37.42	8.28	101.40	41.56	7.80	33.76	67.64	66
		2 e	grams	25.40	25.08	6.126	104.81	49.32	41.04	8.28	96.53	34.46	6.31	28.15	68.38	70
Corn silage.	13	4 w	grams	23.13	24.27	6.126	104.81	43.99	38.69	5.40	99.41	50.08	9.20	40.88	58.53	53
		6 w	grams	23.34	24.49	6.685	104.81	37.09	34.62	2.17	102.64	36.23	6.80	32.43	70.21	58
Do.	14	1 e	grams	33.34	33.57	6.746	55.22	43.41	47.46	55.22	16.74	16.74	14.25	2.49	52.73	95
		3 w	grams	33.79	34.47	55.64	41.12	41.83	41.83	55.64	14.20	10.31	10.31	3.89	51.75	93
Do.	14	5 e	grams	24.95	24.72	4.608	38.10	29.65	34.10	1.18	38.10	11.14	8.33	2.81	35.29	92
		2 e	grams	29.48	29.03	6.507	60.67	45.40	44.90	.50	60.17	9.04	6.61	2.43	57.74	95
1 part alfalfa hay, 3 parts corn silage.	13b	4 w	grams	33.57	36.29	8.168	75.17	50.40	52.28	1.18	75.17	15.95	11.95	4.00	71.17	94
		6 w	grams	27.69	28.12	7.002	64.81	41.79	40.61	1.18	63.63	15.26	10.60	4.06	58.97	91
Do.	14b	1 e	grams	28.03	28.58	7.891	123.74	47.97	69.44	123.74	103.74	12.15	12.15	30.57	135.12	82
		3 w	grams	34.93	34.93	10.521	164.99	29.16	69.44	104.69	104.69	40.22	10.55	24.75	83.24	81
Lined oil sugar, starch, wheat straw.	15	5 e	grams	26.53	26.86	7.014	109.99	15.43	68.05	106.99	106.99	58.24	8.49	24.75	83.24	77
		2 e	grams	27.69	28.12	27.90	9.721	60.05	68.05	131.95	131.95	20.77	6.05	14.72	117.23	83
Do.	15	4 w	grams	34.02	34.02	28.58	134.32	67.66	52.28	7.83	136.40	30.99	12.04	18.05	117.54	80
		6 w	grams	26.31	26.08	7.680	93.96	48.07	44.64	4.33	94.43	27.63	11.14	13.89	78.54	83
Do.	16	1 e	grams	26.31	26.04	2.924	3.67	18.50	18.50	4.33	10.54	10.54	8.58	4.06	58.97	80
		3 w	grams	20.39	20.73	2.736	4.81	20.15	20.15	4.33	10.54	10.54	8.58	4.06	58.97	80
Do.	16	5 e	grams	20.87	17.69	2.441	3.06	20.15	20.15	4.33	10.54	10.54	8.58	4.06	58.97	80
		2 e	grams	20.41	19.73	2.862	3.77	20.34	20.34	4.33	10.54	10.54	8.58	4.06	58.97	80
Do.	16	4 w	grams	25.53	25.86	2.722	4.26	18.00	18.00	4.33	10.54	10.54	8.58	4.06	58.97	80
		6 w	grams	19.96	19.50	3.218	4.62	18.52	18.52	4.33	10.54	10.54	8.58	4.06	58.97	80

A study of the biological values in the last column of Table 5 shows some rather wide variations. For instance, in trial 7, during which sunflower silage was fed, the value obtained for lamb 3, a wether, was 61, whereas the values for the two ewe lambs in the same trial were 84 and 84. Similarly, in trial 8, where the same ration was used, a value of 34 was secured for a wether and values of 77 and 76 for a ewe and wether, respectively. Careful rechecking of the data fails to disclose any discrepancies. These differences must be due to a lower utilization of the proteins by the lambs during these 10-day periods, for the records show that these lambs were normal in every respect while the trials referred to were in progress.

Similarly, in trials 10, 8, and 12, low results were secured with wether lamb 4. However, in subsequent trials the results with this lamb appear to compare fairly well with those for others in the same group.

SUPPLEMENTARY EFFECTS OF PROTEINS FED IN MIXTURES

It has been pointed out that the biological value of a single protein is determined by its amino acid constitution, and that the amino acid present in the minimum quantity, or perhaps its absence, limits the usefulness of that particular protein for production, particularly if the amino acid that is lacking is one which can not be synthesized by the animal body and one which is needed in large amounts for that particular type of production. Adding another protein especially high in the missing amino acid generally results in a biological value of the mixture higher than a mathematical average based on the percentage of nitrogen that each protein contributes to the mixture. This is due to the supplementing effect of the added protein.

In this study alfalfa hay was fed alone and its biological value determined. Similar data were secured for corn silage and sunflower silage. Then alfalfa was fed in the proportion of 1 part to 3 parts of one of the two silages.⁷ The biological values of the single feeds as well as of the mixtures were determined. The results are contained in Table 6.

It is very evident that the dry-matter intake during periods of nearly nitrogen-free rations is, with one exception, lower than during the intervening periods. (Table 5.) This is largely due to the unpalatable nature of this ration. For ideal comparisons it would have been desirable to secure a larger dry-matter intake. However, the rations during trials 5, 6, 15, and 16 contained starch, sugar, and oil, and were much higher in energy per gram of dry matter than the roughage rations fed during intervening trials. It was more important to secure an intake of energy well in excess of the net energy required for maintenance than to increase the dry-matter intake. This was accomplished.

The percentage of crude fiber in the nearly nitrogen-free rations approximates that of periods 9, 10, 13b, and 14b, upon which is based the conclusion of a supplementary effect between alfalfa and corn-silage proteins. This excludes any error that might be due to differences in crude-fiber content of the rations.

⁷ These proportions were chosen because they appear to be those commonly used under practical conditions where silage is readily available.

TABLE 6.—*The biological values of proteins of alfalfa hay, corn silage, and sunflower silage, when fed to lambs singly and in mixtures*

Ration	Trial No.	Lamb No.	Dry matter consumed in 10 days	Protein in feed consumed	Fiber in feed consumed	Observed biological value ^a	Biological values—			
							Calculated	Average observed	Average calculated	Difference
Nearly nitrogen free ^b	5	1 e.....	Grams 1,502	Per cent None.	Per cent 15.99					
		3 w.....	4,155	0.66	15.99					
		5 e.....	2,176	.66	15.99					
Do.....	6	2 e.....	1,868	.63	15.99					
		4 w.....	2,908	.41	15.99					
		6 w.....	2,341	.75	15.99					
Sunflower silage.....	7	1 e.....	2,644	1.52	7.13	85				
		3 w.....	3,644	1.52	7.13	58				
		5 e.....	3,459	1.54	6.78	83				
Do.....	8	2 e.....	3,522	1.55	6.03	77				
		4 w.....	2,445	1.53	5.34	29		67		
		6 w.....	3,706	1.52	6.44	70				
1 part alfalfa hay, 3 parts sunflower silage. }	9	1 e.....	5,944	4.38	12.09	57	66			
		3 w.....	5,998	4.34	12.03	55	53			
		5 e.....	5,670	4.50	12.46	66	63			
Do.....	10	2 e.....	6,126	4.37	12.18	70	70			
		4 w.....	6,126	4.37	12.18	58	38	62	58	4
		6 w.....	6,126	4.37	12.18	68	61			
Alfalfa hay.....	11	1 e.....	10,632	12.63	34.20	59				
		3 w.....	9,953	12.31	34.50	52				
		5 e.....	7,502	12.75	34.12	56				
Do.....	12	2 e.....	10,099	12.38	34.33	67				
		4 w.....	10,411	12.13	34.57	44		56		
		6 w.....	7,808	12.13	34.57	58				
Corn silage.....	13	1 e.....	6,685	1.61	5.57	95				
		3 w.....	6,746	1.61	5.64	93				
		5 e.....	4,608	1.67	5.74	92				
Do.....	14	2 e.....	6,507	1.68	6.06	95				
		4 w.....	8,163	1.68	6.30	94		94		
		6 w.....	7,002	1.68	6.19	92				
1 part alfalfa hay, 3 parts corn silage. }	13	1 e.....	7,891	4.29	13.05					
		3 w.....	10,521	4.29	13.65	81	62			
		5 e.....	7,014	4.29	13.12	77	63			
Do.....	14	2 e.....	9,721	4.29	12.21	88	74			
		4 w.....	9,112	4.29	12.21	80	55	81	64	17
		6 w.....	7,680	4.29	12.38	83	67			
Nearly nitrogen free ^b	15	1 e.....	2,924	.70	13.30					
		3 w.....	3,736	.72	15.10					
		5 e.....	2,441	.64	12.83					
Do.....	16	2 e.....	2,862	.68	15.07					
		4 w.....	2,722	.87	15.07					
		6 w.....	3,218	.83	15.07					

^a Computed by the linear method; see footnote ^b Table 5.^b These rations consisted of raw linseed oil, cornstarch, cane sugar, and selected stems of Albit wheat straw.^c Percentage of fiber in ration fed.

The full data summarized in Table 6 show the biological values of alfalfa hay, corn silage, and sunflower silage when fed singly and in combination.

The silages made of corn and sunflowers are so similar in composition that the daily rations of lambs fed solely these succulent feeds are almost identical in their protein and fiber content. This is brought out in Table 6. Because of this similarity the biological values of the proteins in these two roughage rations may be compared directly. An average value of 67 for sunflower silage as compared with 94 for corn silage shows the superiority of the proteins of corn silage over those of sunflower silage. If one very low value secured with sunflower silage is omitted from the average, a higher average of 75 is obtained.

Similarly, mixtures of 1 part alfalfa hay with 3 parts of either corn or sunflower silage have practically the same protein content and a very similar fiber content. The biological value for the proteins of the alfalfa-sunflower silage mixture is 62. A calculated average, based on the percentage of nitrogen that each protein contributes to the mixture, and by the use of biological values determined when these feeds are fed singly, gives a value of 58. Thus only a slight supplementary effect is noted.

However, in the case of the alfalfa hay-corn silage mixture, the observed average biological value is 81 as compared with 64 for the computed average. There is a marked supplementary effect in the case of this latter mixture. A distinct rise in value of the proteins in an alfalfa-corn silage mixture is noted, whereas no such effect was found in the alfalfa hay-sunflower silage mixture.

NET-PROTEIN VALUES

The loss of nitrogen sustained by an animal on a ration containing sufficient energy but free from protein and other forms of nitrogen is assumed to be the measure of the protein minimum for maintenance.

It can not be concluded that an equivalent amount of digestible protein will cover this requirement. In a similar manner, the protein content of added tissue or of milk secreted does not measure directly the quantity of digestible protein required for their elaboration. There is a wastage of digestible protein in supplying body needs, just as there is a wastage of available energy. The wastage of protein seems to be due to differences in the chemical structure of the feed protein and of the product being formed, as pointed out by Mitchell and Villegas (10).

A determination of food protein required for a specific purpose must not only be based upon the quantity of protein that the body needs, but also on the wastage of food protein in filling this need. The wastage of proteins differs among different food proteins, and a measure of the nutritive value of any protein must include not only wastage due to partial digestibility, but also the wastage of digestible material, representing amino acids, left over in feed protein conversion to body protein or its utilization in the maintenance of body tissue. These left-over amino acids, though available for energy or energy storage, can not be used for meeting the protein needs that arise from lack of the proper supplementary fragments.

The biological value of a protein seems at present to be the most useful means of measuring the nutritive value of digestible protein.

By the use of average percentages of total protein, average digestion coefficients, measuring the wastage of protein in digestion, and average biological values, measuring the wastage of protein in metabolism, it is possible to compute what Mitchell (10) calls the "net-protein" value of feeds. Rather than use the biological value of feeds determined singly in computing the net-protein values of mixtures as pointed out by Mitchell and Villegas (10) it appears that the better method is to use, as far as possible, the biological value as determined for a particular mixture in order to eliminate the error due to the supplementing effect of the proteins of one feed upon the protein of another. This method was employed in computing Table 7.

By the use of this method it is of course necessary to determine the biological values of proteins of feeds for the proportions in which the feeds are commonly fed. The difficulty with this method is that there are too many mixtures used in practice to determine values for all. However, it seems highly desirable to determine the values for those most commonly used.

TABLE 7.—*Net protein values of common roughages fed singly and in combination to lambs*

Roughage	Protein in feed consumed	Coefficient of apparent digestibility ^a	Digestible protein	Average biological value of digestible protein	Net protein content
	<i>Per cent</i>		<i>Per cent</i>		<i>Per cent</i>
Alfalfa hay (irrigated)	12.39	68	8.43	56	4.72
Corn silage (Windus white dent)	1.66	27	.45	94	.42
Sunflower silage (Mammoth Russian)	1.53	33	.50	67	.34
1 part alfalfa hay and 3 parts corn silage	4.29	^b 66	2.17	81	1.75
1 part alfalfa hay and 3 parts sunflower silage	4.39	^b 61	2.08	62	1.66

^a Determined in this investigation; each figure is the average of six coefficients of apparent digestibility.

^b These values have been determined for the mixtures of feeds in column 1 and are not mathematical averages. Weighted averages for the mixtures based on coefficients of apparent digestibility determined by feeding single feeds and weighted in proportion to the quantity of nitrogen each feed contributes to the mixture were computed. The figure for the alfalfa hay-corn silage mixture was 57, being lower by 9 points. For the alfalfa hay-sunflower silage mixture the value was 58, showing a difference of 3 points. These differences between computed and actual values are due to the beneficial effect one feed exerts upon another. It is greatest for the alfalfa hay-corn silage mixture.

An 80-pound lamb, according to Armsby (1), requires 0.044 of a pound of digestible protein for maintenance alone. This quantity of protein can be supplied in the net-protein form by the following quantities of feed: 0.94 pound alfalfa, 2.51 pounds of a 1 to 3 mixture of alfalfa hay and corn silage, or 2.65 pounds of a 1 to 3 mixture of alfalfa hay and sunflower silage. It would be impractical to attempt to supply the protein maintenance requirement of lambs by feeding either of the silages alone, since the bulk would make the ration prohibitive.

Thus, the net-protein value is used in a manner similar to that employed by Armsby (1) in his use of net-energy values.

An 80-pound lamb, according to the maintenance requirement determined in this paper (Table 1), requires 0.053 pound of digestible protein. To meet this requirement, 1.12 pounds of alfalfa hay, 3.03 pounds of a 1-to-3 mixture of alfalfa hay and corn silage, and 3.19 pounds of a 1-to-3 mixture of sunflower silage must be supplied.

Nevens (11) reports that when the corn grain was combined with cottonseed meal or with alfalfa hay the resulting utilization coefficients tended toward a mathematical mean of the utilization coefficients secured with feeding stuffs when fed alone, but were nearer that of the feed other than corn. He concludes that the corn-grain proteins and the alfalfa-hay proteins do not exert any supplementary effect upon each other. The proteins of the whole corn plant, including the stalk, leaf, husk, and kernels, when fed in connection with alfalfa hay, however, show a marked supplementary effect upon each other, as evidenced by the biological values of the proteins when fed singly and in combination, as reported in this paper. The mathematical average of the mixture is considerably below that actually observed.

EFFECT OF DIFFERENT ROUGHAGE RATIOS ON WATER CONSUMPTION AND ON THE URINARY AND FECAL EXCRETION OF LAMBS

The data collected during this investigation afford an excellent opportunity for studying the effect that certain feeds have upon water consumption and upon urinary and fecal excretion. For comparative purposes, results obtained with lambs fed pea and wheat straw, though not discussed in this paper, are included in Table 8 (trials 1 to 4).

Hart and Humphrey (3), working with dairy cows, showed that alfalfa hay has specific diuretic properties, and that its ingestion is generally followed by a marked rise in the output of urine. It is suggested that salts contained in the hay, or possibly specific organic substances, produce this effect.

Table 8 contains the results of eighteen 10-day metabolism experiments during which eight different rations were fed. A study of columns 5 and 6 shows that during trials 5 to 8, 13 to 16, and 18, on an average 32 per cent of the total excretory nitrogen was found in the urine. These trials include the nearby nitrogen-free rations, the straight corn and sunflower silage rations, and a mixture of alfalfa hay and corn silage (trial 18). It forms the only exception in that the ration contained some alfalfa hay and still the urinary nitrogen comprised less than one-half of the total excreted.

The average urinary nitrogen is approximately 59 per cent of the total excretory nitrogen for the groups fed alfalfa hay alone, while a ration of pea straw (Alaska) and one of wheat straw (Albit) alone show a distribution of 49 and 52 per cent. Apparently the partition of ingested nitrogen between the urine and feces is governed to a great extent by the digestibility and percentage of nitrogen in the ration.

Column 13, trials 7, 8, 13, and 14 shows that lambs fed sunflower silage and corn silage alone excreted 3.5 and 0.9 c. c. of urine, respectively, per gram of dry matter consumed. The lambs on sunflower and corn silage rations, drank 1.20 and 0.75 gm. of water per gram of dry matter. This shows strikingly the diuretic effect of sunflower silage.

Column 7 shows the quantity of water that the lambs drank when fed various rations. The data show that when sunflower silage and corn silage alone were fed the water consumption was reduced to a minimum. The averages 191, 158, and 245 gm. of water per 100 gm. of pea straw, wheat straw, and alfalfa hay, shows that the higher protein roughages stimulate thirst. Other factors determining the water consumption of lambs are temperature and humidity. The salt consumption was the same for all lambs, so this factor may be disregarded.

TABLE 8.—*Summary of water consumption, and feces and urine excreted by lambs as related to the type of ration fed*
 [Totals are for 10-day experimental period and per head]

Trial No.	Ration	Lamb No.	Total food eaten		Total dry matter consumed		Total water consumed per gram of food		Total feces		Feces per 100 grams food consumed		Total volume of urine		Urine per gram of dry matter consumed		Nitrogen in urine		Nitrogen in feces	
			Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	C. c.	Per cent	C. c.	Per cent	Per cent	Per cent	Per cent	Per cent
1	Pea straw	1 e.	4,520	1.61	4,519	1.61	9,939	2.02	6,718	1.49	156.94	1.49	6,500	1.32	1.44	1.44	48.26	5.74	5.74	5.74
		3 w.	3,955	1.47	3,715	1.38	7,416	1.88	4,418	1.19	111.71	1.19	8,300	2.13	2.26	2.26	51.93	48.17	48.17	48.17
		3 e.	5,754	2.12	5,171	1.72	9,872	1.72	5,328	1.08	92.60	1.08	3,500	.96	1.06	1.06	32.99	47.01	47.01	47.01
2	do.	12 e.	4,245	1.56	3,987	1.46	9,086	2.35	4,770	1.19	112.37	1.19	7,000	1.65	1.75	1.75	51.98	48.02	48.02	48.02
		14 w.	6,000	2.22	5,382	1.98	10,102	1.68	7,930	1.47	131.67	1.47	9,300	1.55	1.73	1.73	47.54	52.46	52.46	52.46
		16 w.	4,151	1.52	4,702	1.81	9,340	1.81	6,967	1.48	133.26	1.48	10,000	1.94	2.13	2.13	39.80	60.10	60.10	60.10
3	Wheat straw	1 e.	2,324	0.86	2,193	0.80	5,260	2.08	2,824	0.73	111.89	0.73	9,500	3.76	4.33	4.33	66.50	33.50	33.50	33.50
		13 w.	3,816	1.41	3,284	1.22	6,666	1.75	5,787	1.26	151.65	1.26	5,500	.92	1.07	1.07	45.27	54.73	54.73	54.73
		15 e.	2,593	0.96	2,263	0.85	3,381	1.30	3,730	1.65	143.85	1.65	5,500	2.12	2.43	2.43	48.94	51.06	51.06	51.06
4	do.	12 e.	1,590	0.59	1,461	0.54	3,800	2.39	2,305	0.58	144.97	0.58	4,000	2.52	2.74	2.74	57.76	42.24	42.24	42.24
		14 w.	3,590	1.33	3,121	1.15	3,881	1.08	4,340	1.30	120.94	1.30	5,000	1.89	1.60	1.60	51.84	48.15	48.15	48.15
		16 w.	3,273	1.21	2,864	1.07	2,836	.87	3,620	1.26	110.60	1.26	3,000	.92	1.05	1.05	44.03	55.97	55.97	55.97
5	{ Oil, sugar, cornstarch, wheat straw.	1 e.	1,614	0.60	1,502	0.56	2,808	1.74	2,962	0.82	183.52	0.82	7,000	4.33	4.66	4.66	49.07	50.93	50.93	50.93
		3 w.	4,591	1.71	4,155	1.55	5,889	1.28	5,061	1.22	110.24	1.22	7,000	1.52	1.68	1.68	32.86	67.13	67.13	67.13
		15 e.	2,284	0.85	2,173	0.80	4,819	2.11	2,724	1.25	119.26	1.25	5,500	2.41	2.53	2.53	38.76	61.24	61.24	61.24
6	do.	12 e.	1,960	0.73	1,888	0.69	3,460	1.74	2,503	0.68	125.78	0.68	2,500	1.26	1.34	1.34	32.70	67.29	67.29	67.29
		14 w.	3,152	1.18	2,908	1.08	4,667	1.46	3,989	1.35	123.40	1.35	3,500	1.10	1.20	1.20	32.82	67.17	67.17	67.17
		16 w.	2,548	0.95	2,341	0.87	3,034	1.19	3,751	1.60	147.21	1.60	3,500	1.37	1.50	1.50	24.45	75.55	75.55	75.55
7	Sunflower silage	1 e.	16,800	6.24	3,644	1.35	3,127	.19	3,488	.96	20.76	.96	12,000	.71	3.29	3.29	36.62	63.38	63.38	63.38
		3 w.	16,800	6.24	3,644	1.35	5,878	.35	4,018	1.10	23.62	1.10	15,000	.89	4.12	4.12	42.16	57.84	57.84	57.84
		15 e.	16,354	6.06	3,459	1.26	3,920	.02	2,921	.84	17.86	.84	9,000	.55	2.60	2.60	32.54	67.46	67.46	67.46
8	do.	12 e.	16,085	6.00	3,522	1.28	7,771	.48	5,160	1.47	32.27	1.47	14,500	.50	4.12	4.12	37.22	62.78	62.78	62.78
		14 w.	11,214	4.19	2,445	.90	2,896	.26	2,350	.66	20.96	.66	8,500	.76	3.48	3.48	55.57	44.43	44.43	44.43
		16 w.	16,463	6.13	3,705	1.36	5,175	.31	3,080	.83	18.77	.83	12,500	.76	3.37	3.37	37.24	62.76	62.76	62.76
9	{ 1 part alfalfa, 3 parts sunflower silage.	1 e.	14,794	5.44	5,944	2.18	16,975	1.15	6,144	1.08	41.53	1.08	19,000	1.28	3.20	3.20	57.47	42.53	42.53	42.53
		3 w.	14,931	5.50	5,998	2.20	14,771	.99	5,273	.88	35.32	.88	15,000	1.00	2.50	2.50	56.06	43.94	43.94	43.94
		15 e.	14,087	5.22	5,670	2.07	8,936	.63	5,200	.92	36.91	.92	10,000	.71	1.76	1.76	55.66	44.34	44.34	44.34

10	do	12 e. 4 w. 6 w.	15,000 15,000 15,000	6,126 6,126 6,126	17,862 13,126 14,714	1.19 .88 .98	7,976 5,780 4,960	53.17 38.53 33.07	1.30 .94 .81	15,000 13,000 14,000	1.00 .87 .53	2.45 2.12 2.20	41.13 53.24 51.40	58.87 46.76 48.60
11	Alfalfa hay	11 e. 3 w. 5 e.	11,602 11,300 8,480	10,632 9,933 7,502	34,041 15,918 16,060	3.09 1.41 1.89	15,533 10,972 6,386	141.18 97.10 75.31	1.46 1.10 .85	12,850 9,725 4,700	1.17 .86 .55	1.21 .63 .63	55.19 57.48 57.52	44.81 42.52 42.08
12	do	12 e. 4 w. 6 w.	11,620 12,000 9,000	10,069 10,411 7,808	34,065 35,112 22,141	2.93 2.93 2.46	16,063 13,056 7,865	138.24 108.63 87.39	1.59 1.26 1.01	9,500 14,000 9,000	.82 1.17 1.00	.94 1.34 1.15	53.82 66.45 62.25	46.18 33.55 37.75
13	Corn silage	11 e. 3 w. 5 e.	21,515 21,600 14,852	6,685 6,746 4,608	6,145 4,409 1,621	.29 .14 .11	5,680 4,409 2,543	26.40 20.41 17.08	.85 .65 .55	7,000 6,000 5,000	.33 .28 .34	1.05 .89 1.08	27.83 25.67 27.31	72.17 74.33 72.69
14	do	12 e. 4 w. 6 w.	22,672 27,560 24,155	6,507 8,168 7,002	7,559 7,565 3,332	.33 .27 .14	6,679 3,554 3,653	29.46 19.86 15.04	1.03 .68 .62	4,700 6,700 6,200	.21 .24 .20	.72 .82 .89	16.61 24.04 26.75	83.39 73.90 73.25
15	(Oil, sugar, cornstarch, wheat straw.	11 e. 3 w. 5 e.	3,222 4,650 2,723	2,924 3,736 2,441	9,848 5,718 7,122	3.02 1.22 2.62	2,479 3,064 2,780	70.00 78.12 102.09	.85 .98 1.14	3,500 3,000 2,000	1.07 .64 .73	1.20 .80 .82	36.55 24.01 22.94	63.45 73.39 77.00
16	do	12 e. 4 w. 6 w.	3,225 3,000 3,613	2,932 2,732 3,213	8,047 7,640 6,729	2.49 2.59 1.86	2,707 2,845 2,538	85.79 92.57 70.24	.97 1.54 .79	6,000 6,000 6,000	1.86 2.64 2.49	2.10 2.31 2.80	16.05 24.74 33.79	83.05 65.28 66.21
13b	1 part alfalfa, 3 parts corn silage	11 e. 3 w. 5 e.	18,000 20,000 16,000	7,891 10,621 7,014	19,005 13,408 8,112	1.06 .56 .51	6,002 6,198 3,664	33.34 23.82 23.09	.76 .69 .53	10,400 8,225 4,000	.58 .34 .25	1.32 .78 .57	58.00 68.30 31.70	41.91 68.89 31.70
14b	do	12 e. 4 w. 6 w.	24,000 21,000 19,350	9,721 9,112 7,680	18,639 19,252 8,492	.78 .52 .44	5,269 5,234 3,204	21.95 24.62 17.02	.54 .57 .43	10,000 10,000 5,500	.42 .46 .28	1.03 1.10 .72	25.42 31.61 35.24	74.58 68.89 64.76

SUMMARY

Biological values of the proteins in alfalfa hay, corn silage, and sunflower silage were determined. These were found to be 56, 94, and 67, respectively.

A combination of 1 part alfalfa hay and 3 parts of corn silage had a value of 81, while the mathematical mean calculated on the basis of the nitrogen that each contributed to the mixture was 64. This shows a difference of 17, due to the favorable supplementing effect of the two proteins.

Similarly, a value of 62 was obtained for the mixture of proteins contained in 1 part of alfalfa hay and 3 parts of sunflower silage. The mathematical mean of 58 in comparison shows only a negligible supplementary effect.

LITERATURE CITED

- (1) ARMSBY, H. P.
1917. *THE NUTRITION OF FARM ANIMALS*. 743 p., illus. New York.
- (2) FORBES, E. B., and GRINDLEY, H. S.
1923. ON THE FORMULATION OF METHODS OF EXPERIMENTATION IN ANIMAL PRODUCTION. (REPORT OF THE SUBCOMMITTEE ON ANIMAL NUTRITION.) *Bul. Natl. Research Council* 6, Pt. 2 (33), 54 p.
- (3) HART, E. B., and HUMPHREY, G. C., with the cooperation of WILLAMAN, J. J., and LAMB, A. R.
1914. THE COMPARATIVE EFFICIENCY FOR MILK PRODUCTION OF THE NITROGEN OF ALFALFA HAY AND CORN GRAIN. PRELIMINARY OBSERVATIONS ON THE EFFECT OF DIURESIS ON MILK SECRETION. *Wis. Agr. Expt. Sta. Research Bul.* 33: [108]-119, illus.
- (4) KELLNER, O.
1913. *THE SCIENTIFIC FEEDING OF ANIMALS*. Authorized translation by W. Goodwin. 404 p. New York.
- (5) MARTIN, C. J., and ROBISON, R.
1922. THE MINIMUM NITROGEN EXPENDITURE OF MAN AND THE BIOLOGICAL VALUE OF VARIOUS PROTEINS FOR HUMAN NUTRITION. *Biochem. Jour.* 16: [407]-447, illus.
- (6) MITCHELL, H. H.
1923. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. *Jour. Biol. Chem.* 58: 873-903.
- (7) ———
1923. THE BIOLOGICAL VALUES OF PROTEINS AT DIFFERENT LEVELS OF INTAKE. *Jour. Biol. Chem.* 58: 905-922.
- (8) ———
1926. THE DETERMINATION OF THE PROTEIN REQUIREMENTS OF ANIMALS AND OF THE PROTEIN VALUES OF FARM FEEDS AND RATIONS. *Bul. Natl. Research Council* 11, Pt. 1 (55), 44 p.
- (9) ———
1928. A NOTE ON QUANTITATIVE METHODS OF MEASURING THE NUTRITIVE VALUE OF PROTEINS. *Biochem. Jour.* 22: [1323]-1340, illus.
- (10) ——— and VILLEGAS, V.
1923. THE NUTRITIVE VALUE OF THE PROTEINS OF COCONUT MEAL, SOY BEANS, RICE BRAN, AND CORN. *Jour. Dairy Sci.* 6: 222-236.
- (11) NEVENS, W. B.
1921. THE PROTEINS OF COTTONSEED MEAL. II. NUTRITIVE VALUE. *Jour. Dairy Sci.* 4: 552-588.
- (12) ROBISON, R.
1922. THE VALUE OF GELATIN IN RELATION TO THE NITROGEN REQUIREMENTS OF MAN. *Biochem. Jour.* 16: [111]-130.
- (13) SOTOLA, J.
1927. RELATION OF MATURITY TO THE NUTRITIVE VALUE OF FIRST, SECOND, AND THIRD CUTTINGS OF IRRIGATED ALFALFA HAY. *Jour. Agr. Research* 35: 361-383, illus.
- (14) VICKERY, H. B., and OSBORNE, T. B.
1928. A REVIEW OF HYPOTHESES OF THE STRUCTURE OF PROTEINS. *Physiol. Rev.* 8: 393-446.

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No. 2

TEMPERATURE AND SOIL-MOISTURE RELATIONS OF *FUSARIUM OXYSPORUM* VAR. *MEDICAGINIS*¹

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INTRODUCTION

Recent investigations have shown that a disease caused by *Fusarium oxysporum* var. *medicaginis* is present in alfalfa in northeastern Mississippi (9, 10).² Although the distribution of this disease may be quite general, it is definitely known to exist only in that State. It seems very probable, however, that it will eventually be found to occur at least in other Southern States. The seemingly limited distribution of the disease suggested the possibility of a striking reaction to some environmental factor such as temperature or moisture. This led to a study of the response of the causal organism to temperature when growing in pure culture, and of the influence of soil temperature and moisture on infection. The results of these investigations form the basis of this paper.

METHODS

The strain of *Fusarium oxysporum* var. *medicaginis* used in these studies was a pure line of the original isolation and the same as that from which the description was made (10). The effect of temperature on the growth of the mycelium was determined by placing a loop of a suspension of the spores of the fungus on agar in the center of Petri dishes and measuring the rate of growth. The agar was 2 per cent bactonutrient agar plus 2 per cent dextrose, a medium on which the fungus is known to grow luxuriantly. The spores used were obtained largely from sporodochia of a 60-day-old culture growing on potato agar. As soon as the plates were prepared they were placed in incubators held at the desired temperatures, five dishes being used at each temperature.

The effect of soil temperature on infection by *Fusarium oxysporum* var. *medicaginis* was determined by growing plants in temperature tanks similar to those described by Jones, Johnson, and Dickson (6). Each tank was provided with a thermostatic control so that the soil temperature usually did not vary more than 1° or 2°. The temperature of the coldest tank, which ranged from 16° to 18° C., was maintained by running water continuously from the tap through the tank. The soil was prepared by mixing 3 parts of good river-bottom soil

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² Reference is made by number (*italic*) to "Literature Cited," p. 103.

with 1 part of sand and 1 part of fine, well-rotted stable manure. Before the mixture was used it was placed in shallow flats and steamed for four hours without pressure. The moisture-holding capacity of the soil was determined at the beginning of the experiment, and the moisture content was adjusted as desired. The moisture content was maintained by weighing the pots frequently and by adding water as needed. The water was added in each case either to the surface of the soil or through a thistle tube inserted through the hole in the bottom of a 2-inch unglazed flower pot inverted in the soil slightly below the center of the container. The soil moisture could not be kept uniform throughout the pots in this manner, but no better method was known. The surface of the soil was covered with

granulated cork to retard evaporation. The tops of the plants were subjected to ordinary greenhouse temperatures, which ranged from 10° to 30° and averaged about 20°.

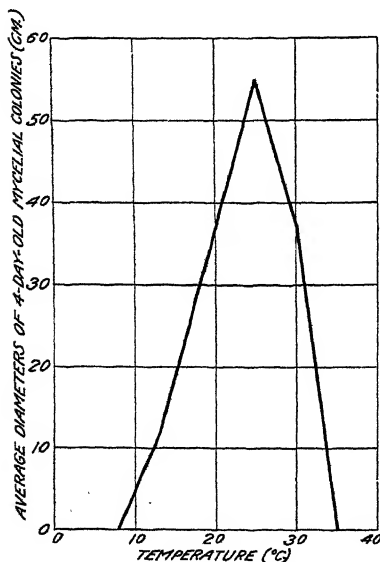


FIGURE 1.—Comparative mycelial growths made by *Fusarium oxysporum* var. *medicaginis* in four days at different temperatures

EFFECT OF TEMPERATURE ON MYCELIAL GROWTH

The temperatures to which the fungus in the Petri dishes was exposed ranged from 3° to 38° C. The comparative rate of mycelial growth in four days at various temperatures is illustrated by the curve in Figure 1. It is clear from this curve that the optimum temperature for mycelial growth lies not far from 25°. There was some growth at 13°, but none at 8°. No macroscopically visible growth was made at 35°. The thermal death point of the fungus lies between 38° and 39°.

The spores germinated, but they made little hyphal growth in three days at a temperature of 37° to 38°. In four days at 8° the germ tubes were about three to five times the length of the spores. None had germinated at 3° in four days, but some of the spores were swollen, indicating that germination would have taken place if it had been practicable to continue the experiment longer.

It is generally known that the cardinal temperatures for the growth of fungi differ somewhat with the strains as well as with the substrata upon which they are growing. Edson and Shapovalov (2) showed that the optimum for the growth of the strain of *Fusarium oxysporum* studied by them when growing on potato agar without sugar was 30° C. Goss (3), on the other hand, found that the two strains of *F. oxysporum* studied by him had their optimum temperatures for growth at 25° and 30°, respectively, when growing on hard potato agar. Link (?) gave the optimum temperature for the growth of *F. oxysporum* on liquid glucose media as about 30°. Both Goss

and Link gave the maximum temperature for the strains with which they worked as near 40°. This temperature agrees very closely with that for *F. oxysporum* var. *medicaginis*.

According to Haskell (4), the optimum temperature for *Fusarium oxysporum* growing in liquid media was 26° to 32° and the maximum was 40° C. Johnson (5) and Massey (8) stated that the optimum temperatures of the varieties of *F. oxysporum* from tobacco and gladiolus were 28° to 30° and 27.5°, respectively.

It seems fairly well established, therefore, that the optimum temperature for the various strains and varieties of *Fusarium oxysporum* lies somewhere between 25° and 30° C.

Edson and Shapovalov (2) pointed out that, of the fungi with which they worked, *Fusarium coeruleum* and *Verticillium albo-atrum* from the northern section of the country had lower maximum and optimum temperatures than *F. radicola* and *F. oxysporum*, parasites from the South. There does not seem to be such a correlation between the geographical source of *F. oxysporum* var. *medicaginis* and its cardinal temperatures, since it is a southern fungus and yet has an optimum for growth as low as or lower than that of any of the strains or varieties of *F. oxysporum* listed above, some of which are from the North. Furthermore, it is quite evident that the cardinal temperatures for *F. oxysporum* var. *medicaginis* when growing in pure culture are such that temperature is not a limiting factor in its distribution in this country.

INFLUENCE OF SOIL MOISTURE AND TEMPERATURE ON INFECTION

Soil moisture, as well as temperature, is often a limiting factor in the prevalence and spread of a disease. To determine whether the amount of *Fusarium* wilt of alfalfa would probably be very greatly influenced by soil moisture, and therefore would be restricted largely to sections of high or low rainfall, two different soil moistures were used in the temperature-tank series run during the winter of 1926-27.

The soil moisture in one half of the cans of each tank was held at 35 per cent and in the other half at 55 per cent of the water-holding capacity of the soil. The alfalfa plants used were of the Kansas Common variety grown from seed sown in the field in August, 1926. The plants were brought to the greenhouse on February 1, 1927, when the experiment was begun, and 10 plants were set in each can. The plants in one of the cans at each moisture and temperature were inoculated by inserting hyphae beneath the bark of the taproot just below the crown, and those of another were inoculated by pouring about the plants 100 c. c. of a very heavy spore suspension. The two remaining pots at each moisture and temperature were held uninoculated as controls, the plants in one being left uninjured and those in the other being injured like those in the wounded-inoculated lot. This made four pots of each soil moisture at each temperature. The temperatures used were 17°, 21°, 25°, 30°, and 35° C. A record of the temperatures was made two or three times a day throughout the duration of the experiment, and the figures given above are the averages of those taken.

When a plant became infected and the disease had developed sufficiently so that there was little doubt as to its origin, the plant

was removed and isolations were made to confirm the diagnosis. It was necessary to remove the infected plants and maintain a record of them, since the disease developed so slowly and irregularly that some plants would have entirely disintegrated before the termination of

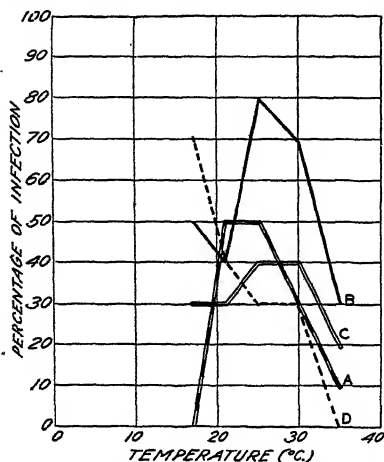


FIGURE 2.—Effect of method of inoculation on percentage of Fusarium infection of alfalfa when growing under different soil-moisture conditions: A and B, inoculum inserted in roots through wounds: A, At 35 per cent soil moisture; B, at 55 per cent soil moisture; C and D, inoculated by pouring a spore suspension about the roots; C, at 35 per cent soil moisture; D, at 55 per cent soil moisture

the experiment (May 9, 1927). The first evidence of infection appeared on February 26 in the can with 55 per cent soil moisture held at 25° C. and inoculated by inserting the fungus in a wound. Subsequently, infected plants appeared from time to time. The percentages of infection at each temperature as influenced by the method of inoculation and soil moisture are presented graphically in Figures 2 and 3.

An examination of the curves in Figure 2 shows that the percentage of infection was greatest when the inoculum was inserted into the root through wounds in the plants growing in a soil with a moisture content equal to 55 per cent of its water-holding capacity. In this case the optimum for infection was 25° C. A comparatively high percentage of plants also became diseased at 17°, the lowest temperature tried.

The high percentage of infection obtained by inserting the inoculum in the host growing in a soil containing 55 per cent water was in marked contrast to that obtained by treating the plants with a spore suspension only. The one notable exception was at 17°. At this temperature 70 per cent of the plants became infected. At 35 per cent soil moisture the difference in the amount of infection was also in favor of the wounding method of inoculation at some temperatures but not at others. For example, at 17°, 30°, and 35° more disease resulted in the unwounded plants, while the reverse was true at 21° and 25°. Judging from these curves, the optimum temperature is not very sharp, ranging from 17° to 30°, depending on conditions. It seems probable that there are too many factors considered in these instances to bring out clearly the effect of temperature.

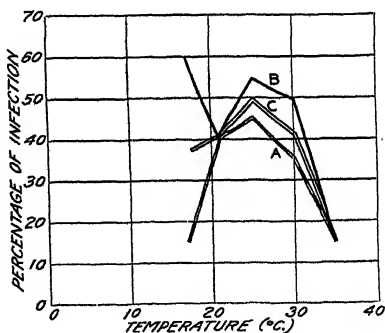


FIGURE 3.—Effect of soil moisture and temperature on percentage of Fusarium infection of alfalfa regardless of the method of inoculation: A, At 35 per cent soil moisture; B, at 55 per cent soil moisture; C, average at both soil moistures

Figure 3 shows the effect of soil moisture and temperature on infection regardless of the method of inoculation, and the average percentage

of infection at each temperature when the other factors are disregarded. These curves have two points in common, namely, their intersections at 21° and 35° C.

They all show an optimum at 25°, although the curve illustrating the infection at 55 per cent soil moisture shows another and even higher optimum at 17°. No explanation for the high percentage of infection at 17° in this case can be seen. It did not occur in the experiment conducted in 1927-28, as is shown in Figure 4. It is clear that 55 per cent soil moisture is more favorable for infection than 35 per cent.

In order to verify the results of the foregoing experiment and to determine whether the influence of temperature varies with the variety of alfalfa used, a similar experiment was run during the winter of 1927-28. The general plan of this test was somewhat different from that run the previous year. Since the 55 per cent soil moisture proved to be the more favorable of the two tried in 1926-27, this one only was used in 1927-28. Approximately the same temperatures were employed as in the previous year. The plants used were of the Hairy Peruvian, an extremely nonhardy variety, and Grimm, one of the hardiest varieties. It was thought that perhaps the disease might react differently on these two varieties, which themselves respond so differently to temperature.

The soil was made up to the desired moisture content, the seeds were inoculated with *Bacillus radicicola*, and one-half of the pots were planted with each variety. After the plants had grown for 39 days, four pots of each variety were placed in each of the five tanks. Three of the pots of each lot were then inoculated by pouring a heavy spore suspension about the plants.

The fourth pot was held uninoculated as a control in each case. The plants were inoculated on November 12, 1927, and the first infection became evident in a Grimm plant on December 1, in the 25° C. tank. On December 12, 10 plants of the Grimm and one of the Hairy Peruvian variety in the 25° tank were diseased. By December 23 a total of 16 plants of the Grimm variety and four of the Hairy Peruvian had become infected at 25°, the optimum temperature. This showed that Grimm was more susceptible, at least at that temperature. Figure 4 shows the percentage of plants of each variety that finally became infected at the different temperatures. Grimm became infected not only earlier but also more heavily at all but the lowest temperature. No disease developed at 35° in either variety. As in the previous experiment, the optimum for infection was near 25°, which coincides also with the optimum for the growth of the fungus in pure culture. The curve obtained the

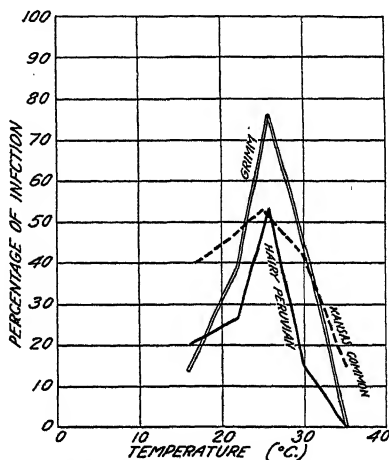


FIGURE 4.—Percentages of infection by *Fusarium oxysporum* var. *medicaginis* in Grimm, Kansas Common var. and Hairy Peruvian alfalfas growing in soil at different temperatures and at 55 per cent soil moisture

previous year when Kansas Common alfalfa was used is also included in Figure 4 for comparison. Kansas Common and Hairy Peruvian gave the same percentage at the optimum temperature, while Grimm was considerably more susceptible under the conditions of this experiment.

These data show that alfalfa may be attacked by *Fusarium oxysporum* var. *medicaginis* over a range of temperatures from 16° to 35° C. The optimum lies somewhere near 25° and the maximum near 35°. The optimum coincides with that for mycelial growth. The minimum temperature for infection was not determined, but it probably is lower than the growing point of alfalfa.

Perhaps a few words about the growth of the different varieties of alfalfa used under the conditions of these experiments would not be out of place. The Kansas Common variety grew more vigorously at a soil moisture of 55 per cent than of 35 per cent. The optimum temperature for the growth of all three varieties was between 20° and 30° C., falling somewhere near 25°. It was noticeable in most instances that the optimum for growth of the alfalfa plants was also the most suitable for their infection by *Fusarium oxysporum* var. *medicaginis* and for the development of the wilt disease. Grimm and Kansas Common made considerably less growth at 16° and 17°, respectively, and at 35° than at temperatures between 20° and 30°. On the other hand, Hairy Peruvian showed a decidedly greater range for vigorous development, for it grew very well throughout the range of temperatures used, as nearly as could be determined by observation alone, although it did seem to show slightly better growth at 25° than at the other temperatures tried. The fact that Peruvian alfalfa will grow over a wider temperature range than the common alfalfa was pointed out by Brand (1).

SUMMARY

A study has been made to determine the effect of temperature upon the growth of *Fusarium oxysporum* var. *medicaginis* in pure culture and upon its ability to attack the alfalfa plant.

The optimum temperature for mycelial growth of this fungus in pure culture is near 25° C., and the maximum is about 37° or 38°. The minimum was not determined, but it lies somewhere below 3°. In general, it may be said that the cardinal temperatures for this fungus do not differ greatly from those for *Fusarium oxysporum*. Although *F. oxysporum* var. *medicaginis* is known to occur only in the South, there appears to be no reason, so far as its cardinal temperatures are concerned, why it should not be widely distributed throughout the country.

Experiments with Kansas Common alfalfa grown in soil at definite soil temperatures and moistures showed that 55 per cent soil moisture was more favorable than 35 per cent for its growth as well as for its infection with *Fusarium oxysporum* var. *medicaginis*. Infection was more certain when the inoculum was inserted beneath the bark of the taproot than when it was added to the soil about the roots. The optimum temperature for infection lies near 25° C., and the disease develops, although poorly, at 35°. The lowest temperature tried was 17°, at which considerable infection resulted. Thus the optimum for infection agrees with that for mycelial growth. Infection takes place

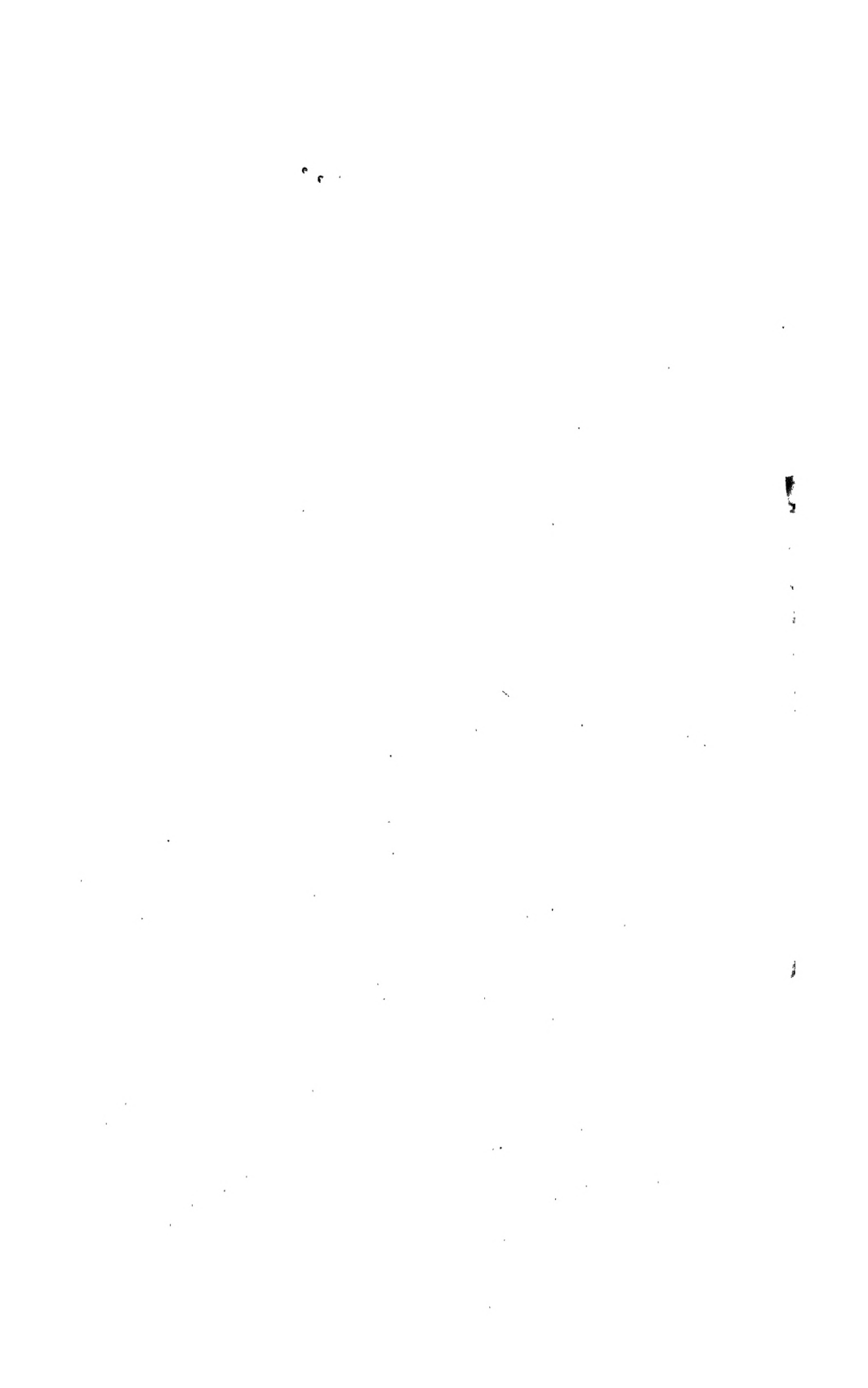
over so wide a range of temperature that this can not be a factor in limiting the distribution of this fungus in this country.

Grimm and Hairy Peruvian alfalfa are also susceptible to the wilt disease, showing about the same optimum temperature for infection. Grimm was slightly more heavily infected than Hairy Peruvian in these experiments.

Hairy Peruvian alfalfa showed a somewhat better growth at the extreme temperatures than did Grimm or Kansas Common.

LITERATURE CITED

- (1) BRAND, C. J.
1907. PERUVAIN ALFALFA: A NEW LONG-SEASON VARIETY FOR THE SOUTH-WEST. U. S. Dept. Agr., Bur. Plant Indus. Bul. 118, 35 p., illus.
- (2) EDSON, H. A., and SHAPOVALOV, M.
1920. TEMPERATURE RELATIONS OF CERTAIN POTATO-ROT AND WILT-PRODUCING FUNGI. Jour. Agr. Research 18: 511-524, illus.
- (3) GOSS, R. W.
1923. RELATION OF ENVIRONMENT AND OTHER FACTORS TO POTATO WILT CAUSED BY FUSARIUM OXYSPORUM. Nebr. Agr. Expt. Sta. Research Bul. 23, 84 p., illus.
- (4) HASKELL, R. J.
1919. FUSARIUM WILT OF POTATO IN THE HUDSON RIVER VALLEY, NEW YORK. Phytopathology 9: [223]-260, illus.
- (5) JOHNSON, J.
1921. FUSARIUM-WILT OF TOBACCO. Jour. Agr. Research 20: 515-536, illus.
- (6) JONES, L. R., JOHNSON, J., and DICKSON, J. G.
1926. WISCONSIN STUDIES UPON THE RELATION OF SOIL TEMPERATURE TO PLANT DISEASE. Wis. Agr. Expt. Sta. Research Bul. 71, 144 p., illus.
- (7) LINK, G. K. K.
1916. A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO. Nebr. Agr. Expt. Sta. Research Bul. 9, 45 p., illus.
- (8) MASSEY, L. M.
1926. FUSARIUM ROT OF GLADIOLUS CORMS. Phytopathology 16: 509-523, illus.
- (9) WEIMER, J. L.
1927. A WILT DISEASE OF ALFALFA CAUSED BY FUSARIUM SP. Phytopathology 17: 337-338.
- (10) ———
1928. A WILT DISEASE OF ALFALFA CAUSED BY FUSARIUM OXYSPORUM VAR. MEDICAGINIS, N. VAR. Jour. Agr. Research 37: 419-433, illus.



A PHYSIOLOGICAL METHOD OF DISTINGUISHING *CRONARTIUM RIBICOLA* AND *C. OCCIDENTALE* IN THE UREDINIAL STAGES¹

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INTRODUCTION

A differential investigation has been undertaken to separate physiologically the white pine blister rust (*Cronartium ribicola* Fischer) and the piñon blister rust (*C. occidentale* Hedge., Beth., and Hunt (7)³), which are morphologically very similar, on species and horticultural varieties of Grossulariaceae. This study was necessary because the uredinial stages of the exceedingly detrimental white pine blister rust and the native piñon or nut pine blister rust, which economically has little importance, are macroscopically indistinguishable, and because both rusts occur in the Pacific Northwest where a control campaign is now being conducted against the recently introduced white pine blister rust, which threatens the highly valuable stands of western white and sugar pines (*Pinus monticola* D. Don. and *P. lambertiana* Dougl.) in the forests of that region. The biometric method recently described by Colley (4), by which the uredinial stages of *C. ribicola* and *C. occidentale* in most cases can be separated on the basis of differences in the average lengths and wall thicknesses of the urediniospores, has been shown to be fairly good, but fallible because some specimens are so near the biometric border line separating the two species that their identity became dubious.

To obtain differential hosts, many species and horticultural varieties of foreign and native Grossulariaceae were assembled for propagation. The species of *Ribes* herein reported include the foreign species and varieties, a group in which the best differential hosts were discovered. Differential results with a group of *Ribes* from the Pacific Northwest have been reported (5).

HOST PLANTS TESTED

The foreign species and varieties of *Ribes* tested include horticultural varieties of the common red and white garden currant, horticultural varieties of *R. nigrum* L., and miscellaneous species.

The considerable number of varieties of the common garden currant tested in these experiments have been grouped for convenience under

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² The writer is under obligation to members of the Office of Forest Pathology for advice and assistance in conducting this investigation; to the late C. S. Sargent, formerly of the Arnold Arboretum, Jamaica Plain, Mass.; to Paul Thayer, of the Ohio Agricultural Experiment Station, Wooster, Ohio; to R. E. Horsey, of Rochester Park, Rochester, N. Y.; to W. H. Alderman, of University Farm, St. Paul, Minn.; to George M. Darrow and Miss M. R. Newman, of the Office of Horticultural Crops and Diseases; to George C. Hedgecock, of the Office of Forest Pathology, for *Ribes* material for inoculating purposes; to E. P. Meinecke, of the latter office, to J. S. Boyce and the late Ellsworth Bethel, both formerly of that office, and to members of the Office of Blister Rust Control, for culture strains of rusts; and to Carl Hartley, of the Office of Forest Pathology, for valuable assistance in the preparation of the manuscript.

³ Reference is made by number (italic) to "Literature Cited," p. 120.

the name *Ribes sativum* (*R. vulgare*) until they can be assigned to the proper species. Bunyard (2) in his study of this group concluded that certain varieties sprang from the following species: *R. vulgare* Lam., *R. petraeum* Wulf., and *R. rubrum* L. This opinion was also held by Wilmott (15) and Thayer (13, 14). To quote the latter (14, p. 316):

It so happens that the first of the species [*R. vulgare*] given * * * in which we are interested is historically first so far as introduction is concerned and also first in commercial importance, for there are but few varieties in general cultivation that do not show the influence of this species.

On the other hand, Berger (1) and other authorities considered *R. sativum* (Rchbch.) Syme the preferred name for the garden currant. According to Berger, the subgenus representing the group of red currants comprises 15 more or less closely related species, natives of the Northern Hemisphere; *R. sativum* occurs in western Europe and in North America, where it has escaped from cultivation and is subsontaneous from Massachusetts to Ontario and Wisconsin, south to Virginia, and in Oregon and British Columbia. For the most part the varieties studied in these differential tests were obtained from nurserymen; hence certain of the varietal names herein quoted can not be taken as thoroughly dependable because of the possibility that varieties obtained from this source may not always be true to name.⁴ Certain varieties are still to be assigned their proper designations. Where names of varieties were uncertain or not found listed, the writer has indicated these facts by quotation marks.

Carefully named varieties of red and white currants were obtained from the following sources:⁵ From Paul Thayer: 11 B, London Market; 14 A, Victoria; 28, Holland; 51 B, Wilder; 58 B, White Dutch; 67 B, Transparent. From George M. Darrow: White Versailles, Goliath, and Holland. A variety cited as "Ginkgoides" is a new unnamed variety which originated in the garden of the late Ellsworth Bethel, Denver, Colo. Bethel regarded it as a sport or variety of the common garden red currant.

Horticultural varieties of *Ribes nigrum* were also tested. The black currant, unlike the red, appears to be derived from a single species which has certain variations, as stated by Hatton (6). The varieties investigated included Champion, "Carter," Victoria, Black Naples, "Black Dutch," Boskoop Giant, and Blacksmith. The last two varieties were received from Paul Thayer.

Other species tested from the following sources included: From C. S. Sargent: *Ribes succirubrum* Zabel, *R. culverwellii* MacFarl., *R. alpestre* Decaisne, *R. tenue* Jancz., *R. carrierei* Schneider, *R. giraldii* Jancz. From R. E. Horsey: *R. carrierei*, *R. fasciculatum* Sieb. and Zucc., and *R. luridum* Hook and Thom. From G. M. Darrow: The hybrid Van Fleet gooseberry (regarded for convenience as *Grossularia reclinata*). From Block Island, R. I.: *R. alpinum* L.

⁴ In attempting to determine the proper names to be applied to each variety investigated by the writer the difficulty just alluded to has been increased by the great confusion that exists in the nomenclature of the common garden currant. The wide distribution of the currant varieties in this country and in Europe, under different names, and the fact that the names sometimes appear, as presented in this paper, in a foreign language, and at other times in the translated form, makes the determination of the correct name exceedingly difficult. There is even further difficulty because of the conflict of opinion among authorities as to priority among the names by which a variety should be known. In general, the names of varieties used in the present investigation are those listed by Thayer (14).

⁵ Cuttings from a number of varieties were received through the kindness of W. H. Alderman. Unfortunately, these cuttings failed in propagation.

SOURCE OF THE INOCULUM

The inoculum used in the tests was derived from various geographical sources. Uredinial culture strains of *Cronartium ribicola* were obtained from aecia occurring on *Pinus strobus* L. from New England (FP⁶ 37073, 38380, 38382, 40300, and 41012), on *P. monticola* from British Columbia (FP 38805), and on *P. monticola* from Scotland (FP 38801). Those of *C. occidentale* were obtained from aecia on *P. edulis* Engelm. from Colorado (FP 36704, 38418, and 41391) and on *P. monophylla* Torrey and Fremont from Nevada (FP 36028, 36922, 38381, and 41365). Uredinial culture strains of *C. occidentale* were also obtained from uredinia occurring on *Ribes aureum* Pursh. from California (FP 36921, 38386) and from Wyoming (FP 38112).

BEHAVIOR OF THE RUSTS IN THE GREENHOUSE

The behavior of the white pine and piñon blister rusts in the uredinial stages in the greenhouse has been described in an earlier paper dealing with the Pacific northwestern *Ribes* (5). The statements made there apply equally well to the present investigation. Leaves of *Ribes sativum* (*R. vulgare*) proved to be susceptible to *Cronartium ribicola* for only a limited period; immature leaves did not become infected, nor did leaves that had commenced to harden. This limited period of receptivity of *R. sativum*, already referred to in the consideration of the closely related wild red currant (*R. triste* Pall.) in the Northwest (5), appears to be a very constant physiological character, bearing out the observation of York,⁷ who found in his greenhouse tests that plants of *R. triste* from the eastern part of the United States did not develop rust infection from *C. ribicola* until the leaves were two-thirds mature.

On *Ribes nigrum*, *Cronartium occidentale* gave an even more characteristic reaction. Repeated trials with this rust showed that it would infect only scantily, or not at all, very young or middle-aged leaves of *R. nigrum*, whereas it infected moderately and even heavily fully matured leaves at the base of the shoot and produced a large number of telia and uredinia during the fall.

Rust infection in the greenhouse proved much more successful in late summer and fall than at other seasons. This was particularly true for *Cronartium ribicola* on *Ribes sativum*, and for *C. occidentale* on *R. nigrum*. These observations corroborate those of Stakman and Piemeisel (12), who reported that *Puccinia graminis* Pers. developed unusually well in late September and early October, a period they found ideal for rust development in the greenhouse; and those of Spaulding (11), who found in his investigation with *C. ribicola* that leaves produced from buds developing in late summer or fall readily become infected.

Trouble was experienced with mildew (*Sphaerotheca mors-uvae* (Schw.) Berk. and Curt.) on certain of the species tested, particularly the varieties of *Ribes nigrum*, making it impossible at times to use the plants for the experiments. Even the very susceptible *R. nigrum* would barely become infected with *Cronartium ribicola* when the leaves were attacked by mildew. Fortunately, *R. sativum* variety Fay (Fay's Prolific), with which these experiments were largely

⁶ Collection number of specimens for study, Office of Forest Pathology, Bureau of Plant Industry.

⁷ YORK, H. H. FIELD STUDIES OF CRONARTIUM RIBICOLA IN THE WHITE MOUNTAINS OF NEW HAMPSHIRE. [Unpublished manuscript.]

concerned, was not affected with mildew. This freedom from mildew shown by the Fay variety was reported by Salmon and Wormald (9) in England; they observed that a severe outbreak of the American gooseberry fungus occurred on Raby Castle, while the Fay variety in the immediate neighborhood remained uninfected.

So far as the writer observed, there was no apparent difference in the length of time required for the first signs of rust infection to appear on susceptible leaves and on resistant ones of the same species or variety. Fertile pustules matured in a shorter period on susceptible leaves than on resistant ones; aborted or abnormal pustules developed after a somewhat longer period of time. Peltier (8) cited a somewhat similar condition as the result of his infection studies with stem rust of wheat.

METHODS

The methods followed in obtaining the results set forth in this paper have already been described (5). As previously stated, these methods were based upon those used by cereal-rust investigators but adapted to the requirements of the *Ribes* host.

PRODUCTION OF INOCULABLE LEAVES BY MEANS OF COLD STORAGE

Since the differential study involved in the main an extensive experimentation with varieties of *Ribes sativum* (*R. vulgare*), it was necessary to have plants with inoculable leaves for all times of the year, particularly in the fall. Normally, *R. sativum* produces a single crop of leaves in the spring, which by fruiting time become fully matured and hardened, thereby being rendered unfit for inoculation purposes. To insure leaves for continuous inoculation purposes, a cold-storage method was used. Plants of *R. sativum* which had passed through their active growing period or were in a dormant condition were sunk in damp sphagnum moss in flats and placed in a cold-storage room where the temperature was kept approximately at the freezing point. Such plants required watering about every three weeks to prevent their drying out. Plants thus kept at low temperature for two or preferably three months break into leaf readily upon withdrawal from the storage room. They should at first be kept in a cool room after repotting in fresh soil and gradually be taken to warmer sections of the greenhouse. Plants so treated will produce leaves within a month or longer, the length of time depending upon the season of the year. Plants placed in storage during late spring or early summer can thus be made available for fall work, and plants kept over winter can be utilized during the early spring months. Inasmuch as field scouting in the Pacific Northwest, where the piñon and white pine blister rusts are to be found, is carried on mainly during the late summer and fall, this procedure for insuring inoculable leaves at this time is an essential part of any method of identifying by inoculation tests the *Cronartium* specimens which the scouts find on *Ribes*.

RECORDING DATA

The inoculated plants were classified by infection types, based on the pathologic symptoms produced by the rusts on *Ribes* leaves. The symbols indicating these infection types and the relative quan-

tity or abundance of uredinia produced on the infected leaves have been fully described in a paper recently published (5). The symbols indicating the types of infection are described briefly as follows:

Resistant types:

Immune—

○ No uredinia formed; hypersensitive or necrotic areas present or lacking.

Resistant—

● Uredinia minute; associated with hypersensitive or necrotic areas.

Susceptible type:

● Uredinia normal size; no hypersensitive or necrotic areas.

The relative quantity or abundance of uredinia produced on the infected leaves was also expressed in the note taking by symbols. They are described briefly as follows:

=, *Trace*.—Uredinia bare trace, or very few in number.

—, *Slight*.—Number of uredinia below normal, scanty.

±, *Moderate*.—Medium production of uredinia; normal infection.

+, *Heavy*.—Infection heavier than medium.

+++, *Very heavy*.—Extremely abundant production of uredinia.

In recording the abundance of uredinia produced on each host species or variety tested, a rating was given the plant as a whole.

CORRELATING DATA

The symbols indicating the abundance of urediniospores on uredinia-bearing leaves of a given *Ribes* species or variety were reduced to a numerical basis and averaged (5, p. 672). Table 1 gives the numerical expression for each symbol.

TABLE 1.—*Mathematical as related to symbolic expression of the abundance of uredinia production*

Symbols for relative abundance of uredinia produced on infected leaves	Equivalent	Range of class ^a	Mid value of class ^a	Abundance of uredinia
=-----	(X)-----	<i>Per cent</i> Less than 5.....	<i>Per cent</i> 2.5	Trace.
-----	× ^b -----	5-35.....	20.0	Slight.
±-----	×× ^b -----	35-65.....	50.0	Moderate.
+-----	××× ^b -----	65-85.....	75.0	Heavy.
+++-----	×××× ^b -----	85-100.....	92.5	Very heavy.

^a The percentage values are rational expressions of the abundance of uredinia production based on the maximum uredinia development on completely infected leaves of *Ribes nigrum*, under favorable conditions, which were taken as a standard. In converting the symbols into numbers, each was assigned the mid value of the class that it represents.

^b Symbols used in previous white pine blister rust investigations. See Spaulding (11), Range percentage and mid values of classes do not apply to these symbols.

In obtaining the averages shown in Figures 1 and 2, the rating for each plant tested was weighted by the number of spore-producing leaves on the plant.

PHYSIOLOGICAL COMPARISON OF CRONARTIUM RIBICOLA AND C. OCCIDENTALE

ON MISCELLANEOUS FOREIGN RIBES SPECIES

In the differential study of the white pine and piñon blister rusts on foreign species and horticultural varieties, a number of miscellaneous foreign species were tested. The study also included 62 plants (367 leaves) of *Ribes nigrum* inoculated with *Cronartium ribicola* and 72 plants (466 leaves) inoculated with *C. occidentale*,

together with 250 plants (1,713 leaves) of the group *R. sativum* (*R. vulgare*) inoculated with the first-named rust, and 344 plants



FIGURE 1.—Results of inoculations with *Cronartium occidentale* and *C. ribicola* on horticultural varieties of *Ribes nigrum*. The black bar shows the average abundance of fertile uredinia of *C. occidentale* for all the leaves inoculated. In averaging, the results on the different plants were weighted according to the number of spore-producing leaves. The figures above the bars show the total number of leaves inoculated. The symbols at the left margin are on a scale on which 100 is the maximum uredinia production for *C. ribicola* on fully susceptible leaves of *R. nigrum*. The shaded bar shows the same for leaves inoculated with *C. ribicola*

(2,364 leaves) inoculated with the latter. The results are given in Figures 1, 2, and 3 and in the discussion following.

R. alpinum. Apparently within the species *R. alpinum* there are strains which differ in susceptibility.

Both *Cronartiums* produced a heavy amount of normal uredinia on *Ribes carrierei* and *R. succirubrum*. A moderate infection of the same type with both rust species was obtained on the hybrid Van Fleet, and a very heavy infection on the Poorman gooseberry varieties, respectively. Spaulding (11) cited Poorman as a moderate host for *C. ribicola*; Clinton and McCormick (3) reported poor

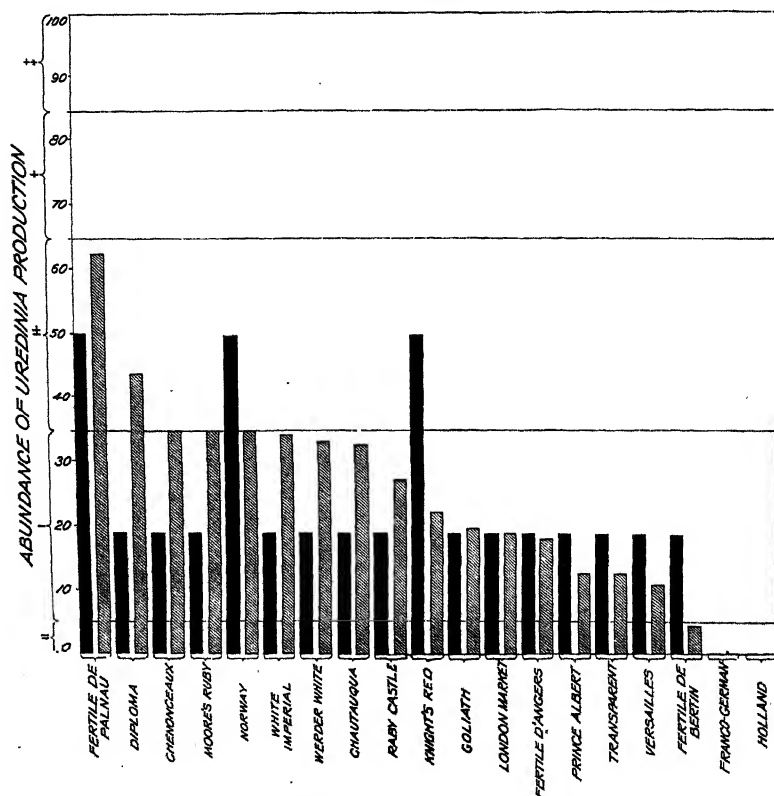


FIGURE 3.—Comparison of results of greenhouse inoculations with *Cronartium ribicola* on horticultural varieties of the group *Ribes sativum* (*R. vulgare*) obtained by the writer and those obtained by Spaulding and his associates. In averaging the writer's results for each variety compared, each plant inoculated was given a mathematical rating equivalent to the mid-point of the degree of infection class to which each belonged. The symbols at the left margin and their conversion to an arithmetical basis are explained on page 116. The shaded bar shows the average abundance of uredinia for the total number of plants inoculated by the writer. The black bar shows the average abundance of uredinia for the total number of inoculated plants reported by Spaulding. The conversion of infection ratings to a basis comparable with the writer's ratings is explained on p. 116.

results on a large gooseberry and Smith (gooseberry) for both *Cronartiums*.

Results on *Ribes fasciculatum* (5 plants, 44 leaves) and *R. tenue* (3 plants, 52 leaves) were negative for *Cronartium occidentale*. On the former host (5 plants, 34 leaves) and on the latter (6 plants, 72 leaves) *C. ribicola* produced a slight (—) infection. The average abundance of uredinia production for these 72 leaves of *R. tenue* gave a numerical value (based on the scale of 100 for completely infected leaves of *Ribes nigrum*) of 6.5. Clinton and McCormick (3)

reported poor plus (P+) results for *R. tenuë* with *C. ribicola* in 10 tests, and Spaulding (11) obtained heavy infection on this host in two tests. Possibly there are also in this instance within the given *Ribes* species strains which differ with regard to rust susceptibility.

ON HORTICULTURAL VARIETIES OF RIBES NIGRUM

In Figure 1 are given the results of a physiological comparison of *Cronartium ribicola* and *C. occidentale* on horticultural varieties of *Ribes nigrum*, a host which is extremely susceptible to *C. ribicola* and which remains highly susceptible throughout the growing season. The European black currant can be regarded as a nurse plant for white pine blister rust. So serious a danger is it to the production of white pine timber that it is now regarded as a definite menace to the white pine timber supply of this country.

Ribes nigrum varieties, Blacksmith and Boskoop Giant, failed to become infected with *Cronartium occidentale*. Infection (type ●) on the other varieties tested with piñon rust was secured on the older fully matured leaves at the base of the shoot. Among the varieties extensively tested, Champion proved to be the most susceptible to *C. occidentale*, but only during the fall period; 73 (27 per cent) susceptible leaves of the 273 tested leaves of this variety produced a moderate amount (\pm) of uredinia. The average uredinia production calculated for these 73 leaves alone was 64.6 (based on the scale of 100 for completely infected leaves of *R. nigrum*). This numerical value was somewhat lower than the value calculated for the amount of uredinia (+) produced by *C. ribicola*, 76.3, for 182 (86 per cent) susceptible leaves out of 211 leaves tested for the same host. Clinton and McCormick (3) obtained only poor results for inoculation of *C. occidentale* on *R. nigrum* and *R. nigrum aconitifolium*; the uredinial stage from *R. gracillimum* on *R. nigrum* failed. Hedgcock, Bethel, and Hunt (7) list *R. nigrum* as one of the poorest hosts for *C. occidentale* in their inoculation tests.

ON HORTICULTURAL VARIETIES OF RIBES SATIVUM (R. VULGARE)

In Figure 2 is shown the comparison between *Cronartium ribicola* and *C. occidentale* with respect to the amount of uredinia produced on varieties of *Ribes sativum* (*R. vulgare*) for the total number of leaves inoculated. With the exception of four varieties—Cerise Incomparable, Cerise Rouge, "Cerise Boisselot," and "Ginkgoides"—on all of which *C. occidentale* produced infections of the resistant type, the other tested varieties of *R. sativum* were apparently quite immune (type ○) to *C. occidentale*. Positive results on *R. vulgare* (*R. sativum*) with *C. ribicola* have been published by Spaulding (11) and by Clinton and McCormick (3). In written communications to the writer, Bethel reported that he had found the "Ginkgoides" variety to be only slightly susceptible to *C. occidentale* under field conditions, and N. Rex Hunt stated that on September 15, 1918, he observed, at Bayfield, Colo., *R. aureum* heavily infected with *C. occidentale*, associated with a red currant almost every leaf of which was infected with the same rust. Hunt did not report the varietal name of this currant or the degree of infection of the rust. He also observed cultivated gooseberry and *Grossularia inermis* (Rydb.) C. and B. infected with *C. occidentale*. Clinton and McCormick (3), as a result of their rust-infection studies of leaves in Petri dishes, and of pot

inoculations, reported negative results for infection with *C. occidentale* on *R. sativum* variety Fay, small currant, and white currant.

The largest number of tests in the present study were made on *Ribes sativum* variety Fay with culture strains of *Cronartium ribicola* from New England and British Columbia on 61 plants (345 leaves). The following culture strains of *C. occidentale* were tested on 100 plants (530 leaves) of the same host variety: One strain from Colorado, on 25 plants (130 leaves); two strains from California (Monrovia), on 29 plants (143 leaves); and three strains from Nevada (Minden), on 46 plants (257 leaves). All the horticultural varieties of *R. sativum* producing fertile uredinia of *C. ribicola* could be classified as belonging to the susceptible type ●. (See p. 110.) Of the 48 varieties tested with *C. ribicola*, 36 produced normal uredinia; 9 produced only resistant-type uredinia; 3 remained immune. The 9 varieties which were resistant (type ○) under the conditions of the inoculation experiments were: "A Fruits Rouge," "Tompelson Rouge," "Ginkgoides," Fertile de Bertin, London Market, Perfection, Red Grape, La Turinoise, and White Dutch.

So far as tested, the three varieties Franco-German, Holland, and Victoria were immune (type ○) to both rusts. Further experimentation may duplicate the results already obtained with these three varieties, which so far have proved immune to both *Cronartiums*, and eventually they may be conclusively regarded as valuable red currants for planting in localities where the growing of this *Ribes* is desired along with that of white pines. Spaulding (11) listed varieties of red currant tested under the following varietal names as resistant to *C. ribicola* but not entirely immune: Eyatt Nova, Franco-German, Holland, London (London Market), Rivers (Rivers Late Red), and Simcoe King. The stock of Franco-German and Holland tested by the writer and found to be immune did not come from the same source as that used by Spaulding.

The three currant varieties listed above by the writer as being immune to *Cronartium ribicola* under the conditions of the foregoing inoculation experiments belong to two of the large groups into which the cultivated currants have been classified (1, 2, 14). The Victoria variety belongs to the group "*Ribes rubrum* and hybrids" and was reported by Thayer (14, p. 386) as being resistant to disease. In this group is also found the London Market (London) variety, which both Spaulding (11) and the writer found to be resistant but not immune.

In the group "*Ribes petraeum* and hybrids" are found the Holland (Long Bunch Holland) and Franco-German varieties, the latter regarded by Thayer (14, p. 391) as synonymous with Holland. Concerning the Holland variety, the chief value of which is in its extreme lateness and great resistance to heat and drought, Thayer stated that in the prairie region this variety had done very well.

Prince Albert (Rivers Late Red), which Spaulding (11) found to be resistant but not immune to *Cronartium ribicola*, is also in the *Ribes petraeum* group. The same investigator reported (11, p. 19) a moderate degree of infection on *R. petraeum*. Until further information is available, it is not wise to favor the cultivation of the Prince Albert (Rivers Late Red) variety, as Thayer suggests (14, p. 394), on account of the seeming resistance of *R. petraeum* to the blister rust, for both Spaulding (11) and the writer have found that

variety to be susceptible (type ●), although only a slight infection (—) was secured. Further experimentation will be necessary, however, before a final, definite opinion concerning the disease resistance of this variety can be obtained.

During a visit to Norway the writer had the opportunity to observe a variety of red currant known as "Red Dutch," which was highly resistant to white pine blister rust. This variety was observed in August, 1927, in the nursery of the Agricultural College at Ås, Norway, growing in immediate proximity to the White Dutch variety, which was heavily infected with the rust. Conditions for rust infection were particularly favorable during 1927 in Norway, and a heavy production of fruiting bodies of species of rusts generally was observed at that time. An examination of plants of the Red Dutch variety did not reveal the presence of sori on any of the leaves. Had the variety been susceptible at all, it is reasonable to believe that it would have become infected during 1927.

Mr. Ivar Jørstad, State mycologist of Norway, Botanisk Museum, Oslo, and Professor Doctor Hagem, of the Botanisk Museum, Bergen, Norway, both regard the Norwegian Red Dutch variety as highly resistant to the white pine blister rust. Jørstad, in correspondence with the writer, has given the following information concerning the synonymy of this variety:

I can inform you concerning the synonymy of the red-currant strains in question. I have conferred with Mr. P. Stedje, leader of the Pomological Experiment Station at Hermansverk in Sogn. He is our best specialist in this matter and has even quite a few American red-currant strains in culture (these he has obtained from Paul Thayer in Ohio).

Mr. Stedje tells me that there is a great confusion concerning the names of one and the same strain. The Norwegian "Rød hollandsk druerips" (i. e., Red Dutch grape currant), which is the one resistant to *Cronartium ribicola*, is not identical with the Danish "Rød hollandsk druerips," but with their "Rød spansk" (i. e., Red Spanish). Our "Rød hollandsk druerips" is further not identical with the American "Red Dutch," neither with Long Bunch Holland nor Victoria, but it is very similar to Prince Albert and to Rivers Late Red (the two latter are possibly identical). Our "White Dutch," which is susceptible to the rust, is, according to Mr. Stedje, not closely related to our "Red Dutch," and the same is the case with the American "Red Dutch." Although the two latter possess the same name, they are entirely different.

From observations and inquiry made in Norway, the writer is inclined to regard the Norwegian Red Dutch variety of red currant as having great possibilities as a horticultural variety, particularly for planting in white pine areas of the United States when the growing of *Ribes* is desired along with that of white pine. Comprehensive artificial-inoculation experiments will be necessary, however, to demonstrate completely the apparent immunity of this particular variety under cultural conditions in the United States, before it can be recommended for use in this country.⁸

Figure 3 shows a comparison between the greenhouse inoculation results published by Spaulding (11) for certain varieties grouped under the name *Ribes vulgare* (*R. sativum*) and those obtained by the writer on the varieties of the same group, of which more than a single plant was tested. To make the results reported by Spaulding

⁸Preliminary inoculation experiments performed in 1929 at the Royal Botanic Garden, Edinburgh, Scotland, in which plants of the Norwegian Red Dutch currant were inoculated with a Scottish strain of *Cronartium ribicola* under rigorously controlled conditions demonstrated the Norwegian variety to be immune to white pine blister rust. See HAHN, G. G. PRELIMINARY REPORT ON A VARIETY OF RED CURRANT RESISTANT TO WEYMOUTH PINE RUST. TRANS. Bot. Soc. Edin. 30: 137-146, illus. 1929.

and his associates comparable with those of the writer, it seemed best to reduce the infection rating given by them to a numerical basis, on a scale in which 100 represents the abundance of uredinia on completely infected plants of *R. nigrum* with *Cronartium ribicola* under favorable conditions. The symbol of a cross within parentheses (×) was interpreted to indicate a degree of infection more than 0 but less than 5 per cent (trace of infection); × for 5 to 33 per cent (slight infection); ×× for 33 to 67 per cent (medium infection); and ××× for 67 to 100 per cent (heavy infection). This interpretation was approved by Spaulding. The midpoints of the classes are therefore as follows: (×), 2.5; ×, 19; ××, 50; and ×××, 83. This method of reducing ratings to a numerical basis is practically the same as that used by the writer in averaging his own data (see p. 109), and the converted results of the two investigations conducted under greenhouse conditions show a reasonable agreement.

Ribes sativum can be considered only a fair host for *Cronartium ribicola*, for it produces fewer uredinia than most *Ribes* species even when infected under the most favorable conditions. Clinton and McCormick (3), in averaging their results for infection of *C. ribicola* on *R. vulgare* (*R. sativum*) in Petri dishes and with pot inoculations, reported the following: On *R. vulgare*, F— in 22 tests; on Fay, F— in 24 tests; on *R. vulgare* (small), F— in 18 tests; on *R. vulgare* (white), F in 15 tests. The symbols used by Clinton and McCormick are as follows: O, failure; P, poor; F, fair; G, good; and E, excellent. To quote these writers:

As a rule poor indicates that fewer than five sori developed. Excellent implies the development of 40 or more on a leaf or leaves in a Petri dish and an even greater total number on the leaves of a plant in a pot. Good and fair are intermediate terms.

So far as a comparison between the Petri-dish method of inoculation of *R. sativum* (*R. vulgare*) and the pot inoculations was concerned, somewhat better results were obtained with the latter method.

IMPORTANCE OF RIBES SATIVUM (*R. VULGARE*) AS A DIFFERENTIAL HOST

A consideration of the foregoing results indicates that among the horticultural varieties of the group *Ribes sativum* (*R. vulgare*), certain varieties tested upon a comparative basis—Chautauqua, Comet, Diploma, Fay, Fertile d'Angers, Goliath, Grosse Rouge de Boulogne, Knight's Red, Marvin Crystal, Raby Castle, Red Cherry, Red Cross, "Ruby Coster," Moore's Ruby, Werder White, White Grape, White Imperial, White Versailles, and Wilder—have shown themselves to be good differential hosts. The most extensive comparative tests were made with the variety Fay (Fay's Prolific). Repeated tests with Fay have shown it to be a most dependable separating host, provided due attention is given to the securing of suitable leaves for inoculation purposes; the variety lends itself readily to propagation both in and out of the greenhouse. Its freedom from mildew and its adaptability to the cold-storage methods above described make it indispensable as a differential host.

Differences obtained on other host species were not definite enough to be relied upon. The attempts to infect *Ribes nigrum*, varieties Blacksmith and Boskoop Giant, with *Cronartium occidentale* should be carried farther before the immunity of these varieties herein reported can be unqualifiedly accepted. The readiness with which

C. ribicola infects species of Grossulariaceae generally has made it impossible so far to find a species which is resistant or immune to *C. ribicola* and not to *C. occidentale*. Whenever *Ribes* species have shown resistance to *C. ribicola*, they have manifested the same reaction to the piñon rust. Where *Ribes* have shown a difference in reaction between the two, this difference has consisted in better production of uredinia by *C. ribicola* than by *C. occidentale*.

METHOD OF DIAGNOSING AN UNKNOWN CRONARTIUM ON RIBES

It has already been demonstrated (5) that the native Pacific northwestern *Ribes* in areas where the two *Cronartium*s are expected to intermingle are generally susceptible to both rusts. Any *Cronartium* found on *Ribes* in Idaho and the adjoining region is therefore an unknown. Upon receipt of such a specimen from the field, slides of the spores of the unknown *Cronartium* are made for measurement and the remainder straightway inoculated upon *Ribes sativum* (*R. vulgare*) variety Fay. *R. aureum*, a congenial host, is inoculated at the same time. Such an inoculation determines the viability of the unknown spores which are to be tested and also is the means of securing a vigorous stock culture for any further investigation which may be necessary. As an added check, duplicate plants of the Fay variety in the same condition of leaf are also inoculated, as a parallel test, with a known strain of *C. ribicola*. This check on the test of the unknown shows whether the leaves of the variety of *R. sativum* are in the right condition for inoculation; a precaution such as has been pointed out is highly desirable with this host. Results should be obtained within 14 days, the length of time necessary for fertile pustules to appear. The interpretation of the results is explained in Table 2.

TABLE 2.—Method of interpreting results of inoculation tests with an unknown *Cronartium* from *Ribes*

[Positive denotes the production of uredinia (type ●) on some of the inoculated leaves; negative denotes absence of uredinia]

Result of—			Interpretation
Inoculations with unknown <i>Cronartium</i>	Parallel inoculations with <i>C. ribicola</i> on <i>Ribes sativum</i> (vul-gare) variety Fay		
On <i>Ribes sativum</i> (vul-gare) variety Fay	On <i>Ribes aureum</i>		
Positive.....	Positive.....	Positive.....	The unknown is <i>C. ribicola</i> .
Negative.....	do.....	do.....	The unknown is <i>C. occidentale</i> .
Do.....	do.....	Negative.....	The leaves of the variety Fay were not in a receptive condition; the test must be repeated on better host material.
Do.....	Negative.....	Positive.....	The spores of the unknown are not viable; no determination possible.

For decisive negative results, it is of course desirable to use several plants in each category, or to repeat the entire test a few days later. If, for example, the infection with *Cronartium ribicola* on Fay is not very abundant, a failure of the unknown on a single plant of the same host might easily be the result of a slight difference in condition of susceptibility between the different plants of Fay; or it might mean that the spores of the unknown were in a less vigorous condition than

those of the known *C. ribicola*; in either case, if there were no replication of the plants in the experiment, obviously it would be unsafe to attempt to name the unknown.

LIMITATIONS OF THE METHODS OF DISTINGUISHING THE TWO RUSTS

The diagnosis of an unknown Cronartium upon a physiological basis under the conditions worked with by the writer is a somewhat slow process when immediate results are required. Good spore material can be identified by the inoculation method in approximately two weeks; but if the material is poor and must first be cultured on a congenial host, e. g., *Ribes aureum*, *R. odoratum* Wendl., or *R. gracillimum* C. and B., to secure a supply of spores for the differential test, an additional two weeks' delay results. The number of conditioning factors also complicate the procedure of diagnosis. As already stated, inoculable leaves must be available and the proper environmental conditions of temperature, humidity, and light supplied. Ordinarily the high temperatures of summer, particularly in the greenhouses, make it difficult to get satisfactory results, unless such temperatures are controlled.

It must also be kept in mind that in the experiments herein reported only a comparatively few culture strains, representing a limited number of geographical sources, of each species were tested. There is the possibility that certain strains of *Cronartium ribicola* might be found which would not infect *Ribes sativum* (*R. vulgare*) var. Fay, the variety investigated most extensively. There is also, of course, the possibility that a strain of *C. occidentale* might be found which would infect Fay.

Other methods of differentiating the two Cronartiums have been studied. For tentative diagnosis the quicker but less certain biometric differential method of Colley (4), discussed in an earlier part of the paper, can be used and supplemented with the physiological test.

It may be noted that neither the physiological nor the biometric method which has been developed for distinguishing the two rusts is of much use for unknown specimens collected late in the season in the Pacific Northwest. Such material commonly shows teliospores only. Both the methods above referred to require the presence of urediniospores. The need of the blister-rust control workers for a method which will enable them to recognize *Cronartium ribicola* whenever and wherever they find it will not be fully met till a method is found of distinguishing between it and *C. occidentale* in the telial stage. G. G. Hedgcock, as a result of long experience with the *C. occidentale*, is able in most cases to distinguish readily by inspection this fungus from *C. ribicola* in the telial stage, both by the color (7) and by the number and vigor of the telial columns. Of 32 specimens of the former and 12 of the latter species, on *Ribes* hosts common to both, he correctly identified all but 2 without seeing the labels. The writer was able to identify correctly by inspection 37 of these 44 specimens. The macroscopic differences between the two rusts in the telial stage are so slight, particularly in weathered specimens, and so difficult to describe, that they are inadequate for general diagnostic use. An effort is now being made in the Office of Forest Pathology to develop a microscopic method of distinguishing between the telia or sporidia of the two species, but no constant difference has thus far been found.

SUMMARY

Foreign *Ribes* species and horticultural varieties were inoculated with a number of strains each of *Cronartium ribicola* and *C. occidentale* in the greenhouse at Washington, D. C., to discover physiological differences between the white pine and piñon blister rusts, which morphologically are very similar in their uredinial and telial stages. In the Pacific Northwest, where a control campaign is now being conducted against the recently introduced serious and menacing white pine rust, workers are faced with the perplexing problem of distinguishing macroscopically this detrimental rust on *Ribes* from the native piñon rust which economically has little importance.

Essential physiological differences were established between the two *Cronartium*s in the uredinial stage under artificial greenhouse conditions. A large number of horticultural varieties of the common garden currant, grouped for convenience under the name *Ribes sativum* (*R. vulgare*) were found to be immune to *C. occidentale* and susceptible to *C. ribicola*. Extensive and thorough tests with the Fay variety (Fay's Prolific) have demonstrated this variety to be particularly adaptable for experimental use as a dependable differential host. More limited comparative tests with other currant varieties indicated similar relations. Of 48 varieties inoculated with *C. ribicola*, all except 3 became infected; of 41 varieties inoculated with *C. occidentale* only 4 became infected, and these to a negligible degree, producing only uredinia of the resistant type.

The three varieties of the group *Ribes sativum* (*R. vulgare*)—Franco-German, Holland, and Victoria—which proved to be immune, so far as tested, to both rusts, may eventually after further examination prove to be valuable red currants for planting in localities where the growing of this *Ribes* is desired along with that of white pines. Further rigorous inoculation experiments will be necessary, however, to demonstrate completely the apparent immunity of these three varieties.

Ribes nigrum, the most receptive of all uredinial hosts to *Cronartium ribicola*, developed only scant infection with *C. occidentale* in the total number of tests made. *C. occidentale* further differed from *C. ribicola* in its reaction on this host, in that it infected fully matured leaves only during the fall period much more readily than it did younger leaves, which were fully susceptible to *C. ribicola*. Of the varieties of *R. nigrum* extensively tested, Champion was most susceptible to *C. occidentale*. In the limited number of tests made the Blacksmith and Boskoop Giant varieties were immune to *C. occidentale*.

Tests with 12 miscellaneous foreign *Ribes* species showed only two *R. tenue* and *R. fasciculatum*, which were susceptible to *Cronartium ribicola* and immune to *C. occidentale*. These two hosts should be further tested to determine the constancy of the very limited differential results obtained.

The procedure for diagnosing unknown uredinial material on *Ribes* from the Pacific Northwest by the greenhouse method is described. The physiological method of differentiating the white pine and piñon blister rusts, performed in accordance with the conditions under which the writer worked, while somewhat slow, appears much more certain than any other method now available of distinguishing *C. ribicola* and *C. occidentale* in the uredinial stages.

LITERATURE CITED

- (1) BERGER, A.
1924. A TAXONOMIC REVIEW OF CURRANTS AND GOOSEBERRIES. N. Y. State Agr. Expt. Sta. Tech. Bul. 109, 118 p., illus.
- (2) BUNYARD, E. A.
1917. A REVISION OF THE RED CURRANTS. Gard. Chron. (3) 62: 205-206, 217, 232, 237, illus.
- (3) CLINTON, G. P., and MCCORMICK, F. A.
1924. RUST INFECTION OF LEAVES IN PETRI DISHES. Conn. Agr. Expt. Sta. Bul. 260, p. [475]-501, illus.
- (4) COLLEY, R. H.
1925. A BIOMETRIC COMPARISON OF THE UREDINIOSPORES OF CRONARTIUM RIBICOLA AND CRONARTIUM OCCIDENTALE. Jour. Agr. Research 30: 283-291, illus.
- (5) HAHN, G. G.
1928. INOCULATION OF PACIFIC NORTHWESTERN SPECIES OF RIBES WITH CRONARTIUM RIBICOLA AND C. OCCIDENTALE. Jour. Agr. Research 37: 663-683, illus.
- (6) HATTON, R. G.
1919-20. BLACK CURRANT VARIETIES—A METHOD OF CLASSIFICATION. Jour. Pomol. 1: [65]-80, [145]-154, illus.
- (7) HEDGCOCK, G. G., BETHEL, E., and HUNT, N. R.
1918. PINON BLISTER-RUST. Jour. Agr. Research 14: 411-424, illus.
- (8) PELTIER, G. L.
1923. A STUDY OF THE ENVIRONMENTAL CONDITIONS INFLUENCING THE DEVELOPMENT OF STEM RUST IN THE ABSENCE OF AN ALTERNATE HOST. II. INFECTION STUDIES WITH PUCCINIA GRAMINIS TRITICI FORM III AND FORM IX. Nebr. Agr. Expt. Sta. Research Bul. 25, 52 p., illus.
- (9) SALMON, E. S., and WORMALD, H.
1921. VARIETAL RESISTANCE TO AMERICAN GOOSEBERRY-MILDEW IN RED CURRANTS. Gard. Chron. (3) 70: 47, illus.
- (10) SCHELLENBERG, H. C.
1923. DIE EMPFÄNGLICHKEIT DER RIBESARTEN FÜR DEN ROST DER WEY-MOUTHKIEFER. Schweiz. Ztschr. Forstw. 74: 25-30.
- (11) SPAULDING, P.
1922. INVESTIGATIONS OF THE WHITE-PINE BLISTER RUST. U. S. Dept. Agr. Bul. 957, 100 p., illus.
- (12) STAKMAN, E. C., and PIEMEISEL, F. J.
1917. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON CEREALS AND GRASSES. Jour. Agr. Research 10: 429-496, illus.
- (13) THAYER, P.
1918. NOTES ON THE NOMENCLATURE AND CLASSIFICATION OF CURRANT VARIETIES. Amer. Soc. Hort. Sci. Proc. (1917) 14: 65-70.
- (14) ———
1923. THE RED AND WHITE CURRANTS—THEIR HISTORY, VARIETIES, AND CLASSIFICATION. Ohio Agr. Expt. Sta. Bul. 371, p. 309-394, illus.
- (15) WILMOTT, A. J.
1918. THE RED CURRANT. Jour. Bot. 56: 19-23, illus.

ALFALFA ROOT INJURIES RESULTING FROM FREEZING¹

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INTRODUCTION

It is generally recognized that winter killing is one of the most common causes of the loss of stands of alfalfa throughout the northern alfalfa-growing sections of North America. Much has been written in recent years on the value of planting only hardy strains of alfalfa as well as on the relation of frequency of cutting and other cultural conditions to the longevity of the stand and the correlation between ~~certain~~ physiological and chemical factors and hardiness. It is not the purpose of this paper, however, to review the extensive literature dealing with winter killing, but rather to record the results of experiments that have a bearing on one phase of this problem.

Not until recently has the attention of investigators been directed to a study of the individual plants that have been injured by freezing. Heretofore it has been customary to consider the stand as a whole; consequently, it has been difficult, if not impossible, to explain the cause of certain types of lesions found on alfalfa roots.

The writer recently described and illustrated (6)³ diseases of alfalfa designated as heart rot and collar rot, which were considered as being directly or indirectly caused by environmental conditions during the winter months. Since then Jones (2) has published a paper in which he discussed from a histological viewpoint the origin of such lesions. Steinmetz (4) illustrated an alfalfa plant showing heart rot due to artificial freezing. Russell and Morrison (3, p. 35) have published pictures of alfalfa plants showing the effects of winter injury. Throckmorton and Salmon (5) showed two plants with lesions just below the crown which they attributed to winter injury. Melchers⁴ also has described a condition that he thought might be due to freezing injury.

Although, with the exception of Steinmetz, the above-mentioned writers attributed the lesions described to winter injury, they did not record having reproduced them. It is the purpose of the present paper to give the results of experiments which were designed to determine the origin of the different types of lesions attributed to winter injury and to learn something regarding the conditions necessary to reproduce them.

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² The writer is indebted to Dr. F. R. Jones for assistance in freezing the plants at Madison, Wis., and in making and interpreting some of the slides used in the histological phase of this work; to Dr. L. R. Jones for the use of the freezing equipment at Madison; and to Prof. S. C. Salmon, of the department of agronomy at the Kansas State Agricultural College, for the use of his freezing chamber.

³ Reference is made by number (italic) to "Literature Cited," p. 142.

⁴ MELCHERS, L. E. CROWN AND ROOT ROT OF ALFALFA (UNDET.). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rpt. 9: 54. 1925. [Mimeographed.]

METHODS

For the most part the plants used in these experiments were of either the Kansas common or the Grimm variety from 6 months to 1 year old. Some of the plants were grown in the greenhouse while others were taken from the field and transplanted to pots in the greenhouse either just before freezing or a month or more prior to their use. In some experiments the plants were frozen in the soil in pots of different sizes, and in others they were removed from the soil before being frozen. In a few instances the plants were frozen just as they stood in the soil in the greenhouse or in the field. In the case of plants removed from the soil preparatory to freezing, they were planted again as soon as they came from the freezing chamber either in soil in pots or in beds in the greenhouse.

Several different types of freezing apparatus have been used. In a number of experiments the freezing chamber belonging to the agronomy department of the Kansas State Agricultural College and directly under the supervision of S. C. Salmon was used. This equipment has been described by Hill and Salmon (1). In other cases the freezing was accomplished by inserting the plants freed from soil in a sheet-iron chamber about 4 by 10 by 16 inches in size, and which was cooled by means of salt and ice. Plants were frozen also by placing over them as they stood in the soil a can containing 100 pounds of ice and various amounts of salt. This can had a raised bottom into which the plants fitted in such a way that they were surrounded by the salt and ice mixture above and on the sides; the bottom of the can rested on newspapers on the ground. In some experiments conducted at Madison, Wis., plants were frozen by placing them in soil in specially designed pots which fitted into artificial ice cans resting in the brine of a refrigerating machine. The temperature was recorded either by thermometers placed near or among the plants as seemed most expedient or with thermographs or thermocouples. Where thermometers were used a record of the temperature was made every few minutes, so that the temperature to which the plants were exposed was known fairly accurately at all times. Plants of each lot were held unfrozen as controls for comparison with those frozen.

Certain other details of methods will be given where pertinent in connection with a discussion of the experimental data.

EXPERIMENTAL DATA

PRODUCTION OF PHLOEM INJURY AND HEART ROT BY FREEZING ALFALFA ROOTS

Several thousand plants have been frozen during the two years that these experiments have been in progress. The plants used differed in the degree of hardening, and the methods were sufficiently diverse so that varying degrees of injury were inflicted. Relatively few winter-injury lesions of the type under consideration developed even among those plants that were more or less severely damaged as indicated by the death of the stems and buds. Only those experiments in which one or more plants developed such lesions will be recorded here. For convenience in discussion the terms "phloem injury" and "heart rot" are used to designate the injury to that part of the root lying outside of the cambium and to that part inclosed by the cambium, respectively. Although these injuries have the

same origin, they do not always appear in the same root; hence it is convenient to have some name by which one or the other may be designated. This usage is consistent with that adopted by Jones (2). The term "phloem injury" as here used is synonymous with collar rot as used in a former paper (6). The frozen plants were examined frequently, some of them being removed from the soil after various intervals of time, while others were allowed to grow for several months before they were finally examined. As was pointed out in a previous publication (6, p. 6), not all of the decay of the center of the crowns and upper part of the taproots of alfalfa plants is due to freezing injury. Plants with more or less hollow crowns and taproots can be found in regions where severe freezing never occurs.

In Table 1 are listed the types of injury produced by freezing 8 to 10 months old Kansas common alfalfa plants which had been growing all summer in the field but which were brought into the greenhouse on December 1, 1926, and set in 8-inch pots, where they grew at temperatures ranging from 60° to 90° F. As they were not used in freezing tests until January and February, they were non-hardened. In no case did any plants that were not subjected to experimental freezing develop symptoms of injury similar to those under consideration. The pots of plants were placed in the freezing chamber belonging to the agronomy department, where they were exposed to various temperatures for different periods of time. After being frozen the plants were held in the greenhouse for several months before the final observations were made. As the temperature of the freezing chamber varied considerably at times, the range as well as the average temperature is given.

TABLE 1.—*Phloem injury and heart rot produced as a result of freezing nonhardened Kansas common alfalfa plants in soil*

["Plants injured" means those that showed phloem injury or heart rot. In many cases most of the plants died and only the indicated numbers showed the types of injury under consideration]

Period of exposure (hours)	Temperature of freezing chamber (°C.)		Period between exposure and observation	Plants frozen	Plants injured	Type of injury
	Range	Average				
5-----	-5 to -11...	-6.6	Months	Number	Number	Slight phloem injury and heart rot. One plant, both heart rot and phloem injury. One plant, phloem injury only.
7-----	-5 to -13...	-8.2	6	10	1	
4-----	-8 to -17...	-11.6	4	10	1	Phloem injury and heart rot.
3-----	-8 to -14...	-11.6	4	5	1	Phloem injury.
6-----	-8 to -17...	-13.8	4	5	5	Do.
7-----	-8 to -20...	-14.5	5	5	1	Do.
4-----	-5 to -21.4	-17	6	10	3	Do.
2-----	-18 to -24...	-21	4	5	3	Do.
3-----	-18 to -24...	-21	4	10	2	Do.
Total-----				65	19	

The data presented in Table 1 show that in these experiments phloem injury was more often produced than heart rot. They also show that both types of injury were produced when the plants were exposed to average temperatures of -6.6°, -8.2°, and -11.6° C. for 5, 7, and 4 hours, respectively. On the other hand, phloem injury alone was evident in plants frozen at average temperatures of -11.6°, -13.8°, -14.5°, -17°, -21°, and -21° for 3, 6, 7, 4, 2,

and 3 hours, respectively. It is apparent that the types of injury under consideration can be produced over a considerable range of temperature and time of exposure. Characteristic phloem injury is shown in Figure 1, A-C. In some cases 100 per cent of the plants developed phloem injury, but more frequently the percentage of injured plants was much smaller. A total of 29.2 per cent of the plants used in these tests eventually showed either one or both types of injury. Why one type of injury was produced in some cases and both types in others is not clear from the tests made. All the plants frozen showed more or less injury to the stems and buds.

In Table 2 are shown the results of freezing plants taken from the same lot as those used in the experiments listed in Table 1, but in



FIGURE 1.—A-C, Phloem injury of Kansas common alfalfa plants produced by artificial freezing. The plants were grown from seed sown in the field in the spring. They were transplanted to 8-inch pots December 1, 1926, and kept in the greenhouse until January 25, 1927, when they were exposed for seven hours to an average temperature of -14.5°C . After that they were kept in the greenhouse until July 1, when the photograph was taken. The blackening of the crown and upper part of the taproot illustrates what is designated in this paper as phloem injury. In some seasons lesions of this type are very common on alfalfa plants following severe winter injury. About one-half natural size

this case the plants were not brought into the greenhouse until they were needed for the freezing tests, which were conducted in February, 1927. These plants were subjected to freezing temperatures while in the field, but they showed no evidence of injury when removed from the soil, nor did the controls after growing in the greenhouse for several months. Two lots of 10 plants each were frozen for $1\frac{1}{2}$ and 3 hours at average temperatures of -14.6° and -14.9°C ., respectively. In these instances the roots were not planted in soil but were exposed to the temperature of the freezing chamber without any protection. The plants exposed for 3 hours were very seriously injured and most of them died. One of the plants which survived showed phloem injury after growing for four months in the greenhouse. On the other hand, although the plants frozen for $1\frac{1}{2}$ hours

were not very seriously damaged, one of them showed some phloem injury. (Fig. 2.)

The last experiment listed in Table 2 illustrates an instance in which phloem injury developed in two out of five plants frozen in soil in 8-inch pots for seven hours at an average air temperature of -14° C.

TABLE 2.—*Phloem injury produced as a result of freezing 9-months-old Kansas common alfalfa plants on February 25, 1927, immediately after bringing them from the field*

*Plants injured" means those that showed phloem injury. In many cases most of the plants died, and only the indicated numbers showed the type of injury under consideration]

Period of exposure (hours)	Temperature of freezing chamber ($^{\circ}$ C.)		Frozen in or out of soil	Period between exposure and obser- vation	Plants frozen	Plants injured
	Range	Average				
1½-----	-13 to -16-----	-14.6	Out-----	Months 4	Number 10	Number 1
3-----	-13 to -17-----	-14.9	do-----	4	10	1
7-----	-8 to -17-----	-14	In-----	4	5	2
Total-----					25	4

In Table 3 are summarized the results obtained by freezing Kansas common and Grimm alfalfa plants under various conditions at Madison, Wis. The plants, which were from spring-sown plots, were brought into the greenhouse at the time the experiments were conducted. Twenty-five plants of each variety were placed in soil in specially constructed cans and lowered into the freezing chambers, in which they were exposed to different temperatures for various periods of time. The plants were then replanted in soil, each variety being placed in a different pot. In some cases the pots were held outside of the greenhouse until danger of freezing became imminent, at which time they were placed in a cool greenhouse. Other pots were held in the greenhouse throughout the growing period. In the last two tests listed the plants were frozen in ice by placing the tops, including the crowns and 1 or 2 inches of the upper part of the taproots, in water, which was then frozen. The temperature of the water or ice was obtained at intervals by means of a thermocouple placed in the water along with the plants. The period of time listed in Table 3 includes that necessary to freeze the water, which



FIGURE 2.—Phloem injury on a taproot from the same lot of alfalfa as that illustrated in Figure 1. However, the plant was removed from the soil and brought into the greenhouse February 25, 1927, just before it was exposed for 1½ hours to a temperature of -14.6° C. After the exposure it was planted in soil in the greenhouse and kept there until June 23, when it was photographed. One-half natural size

temperature of the water or ice was obtained at intervals by means of a thermocouple placed in the water along with the plants. The period of time listed in Table 3 includes that necessary to freeze the water, which

was usually 8 to 10 hours. After the plants had been frozen for the desired length of time the ice was allowed to thaw at room temperature, and the plants were then set in soil in pots. Observations taken after 40 days, and 17 and 32 days in the last two instances, showed that a small percentage of the plants had phloem injury, heart rot, or both. A total of 6.9 per cent of the plants showed these types of injury. Many of the plants in some tests were killed outright, others were uninjured, and still others suffered various degrees of injury, as shown by the condition of their tops. The typical lesions under con-



FIGURE 3.—Phloem injury produced by freezing 5-months-old alfalfa plants in soil for 24 hours at an average temperature of -11° C., in a refrigerating machine at Madison, Wis. The two plants (A) and the three (B) are of the Kansas common and Grimm varieties, respectively. These plants were kept in the greenhouse after freezing for 35 days before being photographed. In most cases the entire crowns and upper parts of the taproots are involved. The plant at the left in B shows a distinct band of dead tissue just below the crown. About two-fifths natural size

of time. After being frozen the plants were set in soil in a bed in the greenhouse. The observations made after 21 and 7 days, respectively, showed typical phloem injury in 15 per cent of the plants. (Fig. 4.) No injury was evident in the unfrozen controls.

Although phloem injury and heart rot have developed occasionally in plants frozen in other experiments, the above-cited examples are sufficient to show something of the range of conditions under which these troubles may develop.

sideration were usually found in the few surviving plants in a pot where most of the plants had died. These lesions were found on both the Kansas common and Grimm plants and were produced when the plants were frozen in the soil or in ice for 24 to 48 hours. (Fig. 3.)

In Table 4 are shown the conditions under which typical phloem injury was produced in 12 out of 80 small alfalfa plants of the Kansas common variety, some of which were grown in the greenhouse and the remainder in the field. These plants were removed from the soil, washed, and placed tops down in a chamber cooled with ice and salt to temperatures ranging from -5° to -15° C. for different periods

TABLE 3.—*Phloem injury and heart rot produced as a result of freezing 5-months-old alfalfa plants immediately after bringing them from the field at Madison, Wis.*

["Plants injured" means those that showed heart rot or phloem injury. In many cases most of the plants died, and only the indicated numbers showed the type of injury under consideration]

Variety	Period of exposure	Temperature of freezing chamber (°C.)		Frozen in soil or in ice	Period between exposure and observation	Plants frozen	Plants injured	Type of injury
		Range	Average					
	<i>Hours</i>				<i>Days</i>	<i>Number</i>	<i>Number</i>	
Kansas common.	48	-8 to -13	-11	Soil....	40	25	1	Phloem injury.
Grimm.....	48	-8 to -13	-11	do....	40	25	1	Do.
Kansas common.	48	-8 to -13	-11	do....	40	25	2	Do.
Do.....	24	-8 to -13	-11	do....	40	25	1	Do.
Do.....	24	-8 to -13	-11	do....	40	25	1	Do.
Grimm.....	24	-12 to -16	-14	do....	40	25	1	Do.
Kansas common.	48	-12 to -16	-14	do....	40	25	1	Do.
Do.....	24	-12 to -16	-14	do....	40	25	2	Heart rot and phloem injury.
Grimm.....	24	-12 to -16	-14	do....	40	25	3	Phloem injury.
Do.....	39	0 to -10	-----	Ice....	17	25	4	Do.
Do.....	27	0 to -11	-----	do....	32	25	2	Do.
Total.....						275	19	

* Temperature in ice as determined by a thermocouple.



FIGURE 4.—Phloem injury developed by freezing in 3-months-old Kansas common alfalfa plants grown at Manhattan, Kans. On November 25, 1927, plants were brought from the field and frozen at temperatures varying from -11 to -15° C. for 15 to 25 minutes before being set in soil in the greenhouse. They were kept in the greenhouse until photographed on December 15, 1927. In most plants the injury is evident at varying distances below the crown. About two-fifths natural size

TABLE 4.—*Phloem injury produced as a result of freezing 3-months-old Kansas common alfalfa plants removed from soil and frozen during 1927-28*

["Plants injured" means those that showed phloem injury. In many cases most of the plants died, and only the indicated numbers showed the type of injury under consideration]

Source of plants	Period of exposure	Temperature (° C.)		Period between exposure and observation	Plants frozen	Plants injured
		Range	Average			
	<i>Minutes</i>				<i>Number</i>	<i>Number</i>
Greenhouse	15	-13.4 to -15.....	-14.2	1 month.....	10	2
Field.....	15	-11 to -13.....	-12	do.....	15	2
Do.....	20	-11 to -13.....	-12	do.....	15	2
Do.....	25	-11 to -13.....	-12	do.....	15	3
Greenhouse.....	40	-5 to -7.....	-6	21 days.....	15	2
Field.....	10	-11.5	7 days.....	10	1
Total.....				80	12

FREEZING PLANTS IN THE FIELD

It was thought that perhaps winter-injury lesions could be produced more readily if the plants were frozen as they stood in the soil by applying the cold from above only, thereby more nearly reproducing natural conditions. With this in mind, a number of experiments were conducted in which plants were frozen as they stood in the field, and in a few instances in the bed in the greenhouse, by placing over them a sheet-iron tank containing different proportions of ice and salt as described under the discussion of methods. The plants were growing in rows, and the chamber covered 10 to 15 plants. Temperatures of from -5° to -15° C. could be obtained in this way. The temperature records were taken by means to two maximum and minimum thermometers whose bulbs were placed among the crowns of the plants, one at the end and the other at the center of the group of plants covered by the chamber. In this manner the crowns and the upper part of the taproots of plants about $1\frac{1}{2}$ years old were subjected to various degrees of cold for different periods of time. The tests were conducted during the summer, when the plants could be most readily frozen and when microorganisms would be most active. (The possible relation of microorganisms to the development of these lesions is discussed later.) Since nonhardened greenhouse plants developed the typical injuries, there seemed to be no reason why this work in the field could not be conducted as well in the summer as in the winter.

The crowns and the upper part of the taproots of many of the plants frozen in these experiments died outright. Usually the plants at each end of the row included beneath the chamber died or were severely injured, while those at the center were only slightly affected, and the plants between them showed various degrees of injury as indicated by the appearance of the tops. In no case, however, was typical phloem injury or heart rot produced. A photograph of a plant frozen in this manner is illustrated in Figure 5. However, in this case the freezing was so severe that the root was frozen entirely through. This was typical of what happened in many cases. As it seemed impracticable to produce by this method the lesions so commonly seen in plants frozen under natural conditions, this type of experiment was discontinued. Perhaps further trials at different seasons of the year might have yielded the desired results.

SOME HISTOLOGICAL CHANGES IN FROZEN ROOTS

Jones (2) recently published a paper giving a detailed discussion of the histological changes in alfalfa roots due to winter injury. His studies were all made on material collected from the field. While conducting the freezing experiments at Madison, Wis., details of which are recorded in Table 3, Jones and the writer studied some of the plants frozen under known conditions in order to compare them with the field material studied previously by Jones. Portions of the taproots just below the crown were fixed, embedded in paraffin, sectioned, and stained by the method described by Jones (2). Plants just taken from the freezing chambers as well as those which had been growing for a month in the greenhouse after being frozen and which

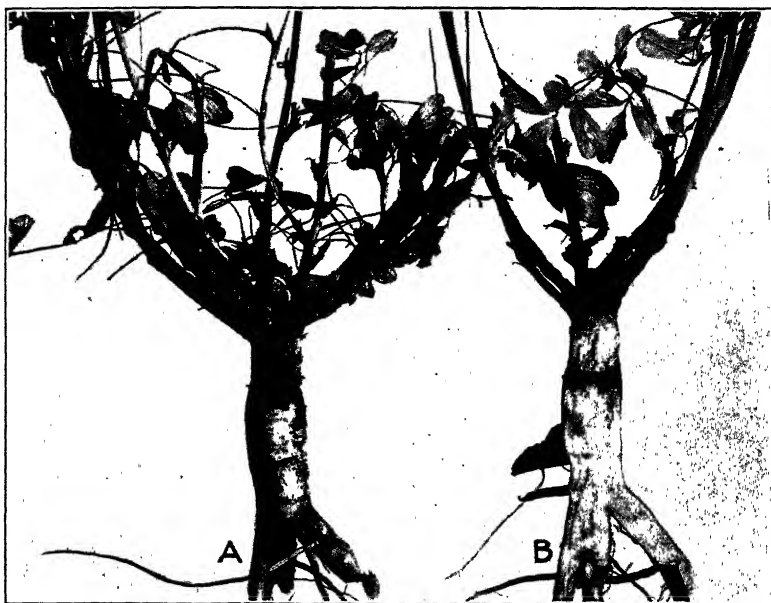


FIGURE 5.—Plant frozen by placing a can containing ice and salt over it as it stood in a bed in the greenhouse. The minimum temperature to which it was exposed was -13.5°C ., and the time of exposure was 23 hours. The crown and the upper part of the taproot were frozen entirely through. The root was halved longitudinally, and the exterior (A) and interior (B) views are shown. About three-fourths natural size

showed typical phloem injury and heart rot were studied. Figures illustrating different types of injuries are given herein.

In Figure 6 is shown a cross section of the root near the crown of a 5-months-old Kansas common alfalfa plant frozen for 24 hours at a temperature varying from -12° to -16°C . (average about -14°) as it appeared when it came from the freezing chamber. The rays are badly split in both the xylem and the phloem, and the phloem parenchyma cells just beneath the exterior cork covering are more or less broken apart. Some roots of the same lot that had been subjected to the same conditions showed few or no rifts in the rays. In some cases the splitting was largely confined to wood rays, while in others only the phloem rays were affected. The amount of splitting also varied greatly. However, in extreme cases both the wood and phloem

rays were split. The cambium cells were frequently collapsed. There was no unusual staining reaction at this time, the sections taking the stain as did healthy tissue of unfrozen control plants, sections of which were prepared and studied at the same time. The splitting off and sloughing away of the outer layer of the upper part of the taproot and crown of young plants when frozen in the field are of very common occurrence. The manner in which such a condition is brought about is evident in the section shown in Figure 6. In some

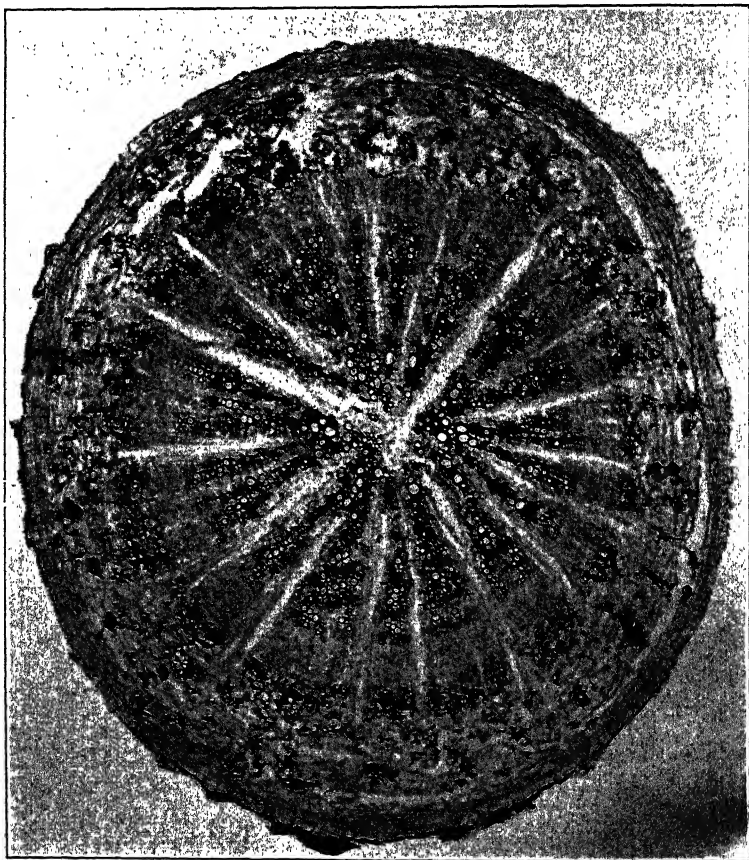


FIGURE 6.—Cross section of a root of a 5-months-old Kansas common alfalfa plant frozen in a pot of soil for 24 hours at an average temperature of -14°C . Note the rifts along the rays (light streaks) as they appeared when the root was taken from the freezing chamber. Compare with sections of roots from the same lot illustrated in Figures 7, A and B, and 8, A. $\times 33\frac{1}{4}$

sections this layer is found to have been entirely loosened from the phloem parenchyma beneath.

In Figure 7, A, is shown a cross section of one of the roots of the same lot as that discussed above. This root had been planted in soil and grown for 40 days before it was photographed. It shows typical heart rot without any apparent phloem injury. A portion of this root was infiltrated with paraffin, sectioned, stained, and photomicrographed. It is reproduced as Figure 7, B, and shows that a

considerable amount of splitting of the wood rays was caused by the freezing. There appears to be some injury in the phloem parenchyma, especially near the side rootlet. Injury in the xylem as well as the phloem is denoted by the deeper stain which has photographed black. The cambium in this plant was to all appearance uninjured, and the cork layer remained intact throughout.

A photomicrograph of another root of the same lot is shown in Figure 8, A. This root also had heart rot as well as phloem injury. Much splitting in the wood rays is evident, and the injured cells

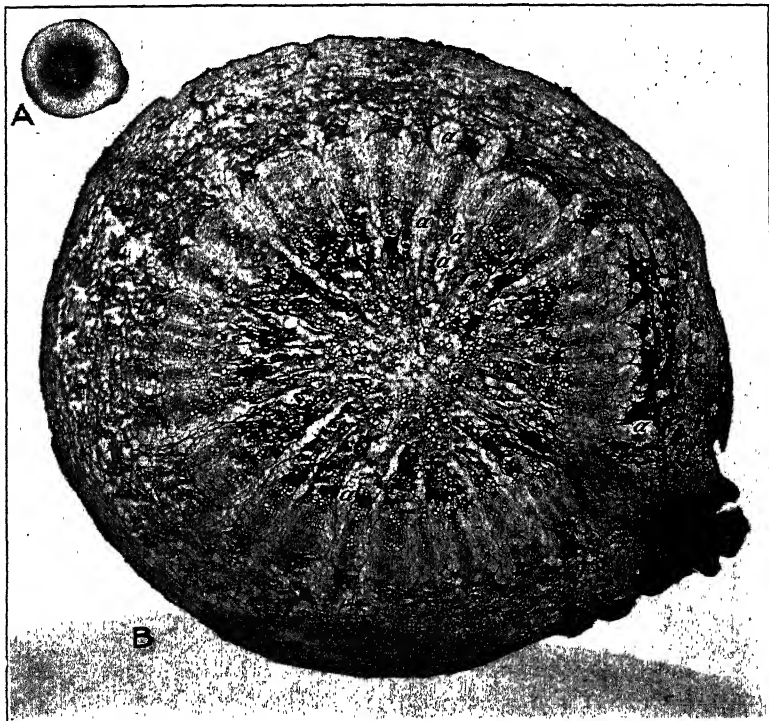


FIGURE 7.—A, Cross section of an alfalfa root from the same lot as that in Figure 6. The plant had grown in the greenhouse for 40 days before being sectioned. Typical heart rot had developed in this root as indicated by its dark center. $\times 5$. B, Cross section of the same root as in A, showing in more detail the structure of the root. The rifts in the wood rays are still evident, and many of the cells have died from the effect of freezing and take the stain more deeply than the living cells. There was also a limited amount of phloem injury near the side rootlet, as indicated by the darker stain. Some of the openings resulting from the freezing, a few of which are indicated by the letter *a*, have been closed by the formation of new cells within them. The outline of the original opening is still evident. Compare with Figure 8, B, which shows similar rifts still remaining open due to the fact that the surrounding cells were killed by freezing. $\times 32$

largely near the cracks took the stain more deeply. The greatest injury in this root is in the phloem, as indicated by the dark-staining area. Many of the phloem cells have collapsed and are badly disorganized. A meristematic layer has been laid down in the phloem which separates the dark-staining injured tissue from the uninjured portion of the phloem nearer the cambium. This layer is not equal in thickness and undoubtedly is not such an effective barrier as was furnished by the cork layer originally present on the outside of the root. The dead tissue outside of this wound periderm was decaying

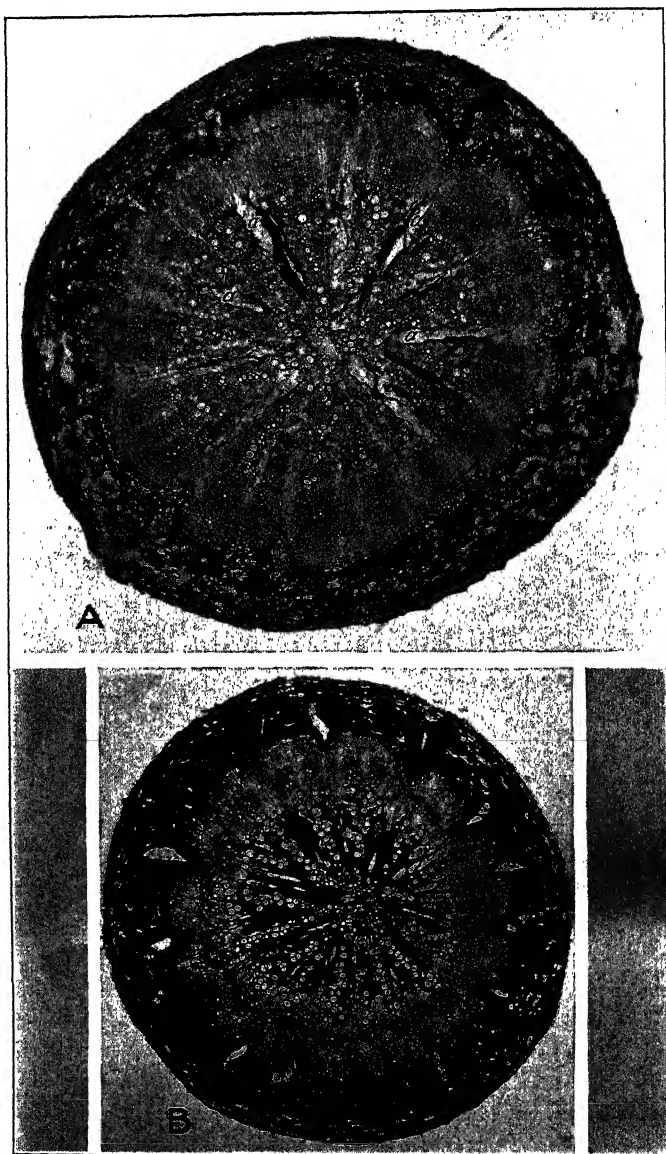


FIGURE 8.—A, Cross section of a root from the same lot of alfalfa as that shown in Figures 6 and 7, A and B, taken 40 days after freezing. Here the phloem seems to have suffered most, as a large part of the cells were killed outright. There is some splitting of the wood rays bordered with dead cells as well as some dead cells in the wood which have stained dark. This figure shows the appearance of the interior of roots affected with phloem injury as illustrated in Figures 3 and 4. Note also the healed cracks at the points indicated by *a*. $\times 32$. B, Cross section of an alfalfa root of about the same age as that shown in A and frozen under natural conditions at Manhattan, Kans., during the winter of 1927-28. Both phloem injury and heart rot followed the splitting of the rays and death of the cells. Much of the phloem was killed by the low temperature, as indicated by the black-staining area, in the outer part of the section. Many of the largest rifts are surrounded with dead (black) cells, and there is little if any evidence of the closing of these openings by the formation of new cells. Considerable meristematic activity that had taken place resulted in the formation of a wound periderm just inside of the dead portions of the phloem. $\times 19$

and would no doubt eventually have sloughed off. In the meantime this dead tissue is open to attack by various soil fungi and bacteria, some of which may be weak parasites, and once having established themselves in the dead tissue, can find their way through the weakened or even incomplete periderm beneath and hasten the death of the plant.

A section of a root showing more severe heart rot as well as phloem injury is shown in Figure 8, B. Here the frost cracks both in the phloem and xylem are surrounded by the deep-staining dead cells. As in Figure 8, A, the inner part of the phloem is separated from the dead outer portion by a heavy meristematic layer. This outer tissue eventually sloughs off, the dead central xylem decays, and the result is a root whose crown and upper part of the taproot form little more than a thin-walled hollow cylinder with a roughened exterior, resembling the root previously illustrated by Weimer (6, *fig. 2, A and B*).

It having been observed that the heart rot and phloem injury were usually found in plants that showed more or less ray splitting and tearing apart of parenchymatous cells, the question arose as to whether such splitting was necessarily associated with the production of such symptoms. To obtain information on this point, roots of plants that had been frozen by different methods were sectioned. It was soon learned that plants removed from the soil and frozen quickly did not always show splitting, at least not to the same extent as did similar plants frozen slowly in soil. Young plants in soil in pots withstood an air temperature of -10°C . or thereabouts (soil temperature not recorded) for 48 hours, but they were killed in 15 to 20 minutes at that temperature when removed from the soil and then frozen. It seems probable that, when the plants are cooled slowly as they are when frozen in soil, large ice crystals are formed between the cells in certain local areas—especially where the cell walls are thin and the water content high, as in the rays and phloem parenchyma—and the cells are broken apart. On the other hand, when freezing is rapid, ice crystals are formed comparatively uniformly throughout the tissue and the cells are killed without the localized splitting and tearing. At least this explanation seems plausible in the light of Wiegand's studies on the formation of ice crystals in plant tissue (?).

ASSOCIATION OF MICROORGANISMS WITH WINTER-INJURY LESIONS

Isolations were made from a large number of plants affected with phloem injury and heart rot from the field and the greenhouse, and usually a species of *Fusarium*, bacteria, or both were obtained. More rarely *Rhizoctonia solani* was isolated. However, the same species of *Fusarium* was seldom obtained from different lesions. No one organism could be isolated very consistently from such decaying tissues. It seemed probable, therefore, that these microorganisms associated with the lesions occurring in roots subjected to freezing were simply saprophytic organisms living in the frozen tissue. This supposition was substantiated by the fact that no such lesions were ever found on unfrozen control plants or plants only slightly frozen.

In order to determine the pathogenicity of some of the organisms isolated, the following experiment was conducted: Young alfalfa plants of the Kansas common variety growing in the greenhouse were

removed from the pots and frozen for 10 and 15 minutes at a temperature of $-12^{\circ}\text{C}.$, and then they were planted in soil in 8-inch pots and held in the greenhouse. The following treatments were used:

(1) Plants frozen for 10 and 15 minutes and unfrozen controls were planted in soil steamed for four hours without pressure and not infested with the organisms being tested. For each lot of plants 4 pots were used, making a total of 12 pots containing 60 plants.

(2) Plants frozen for 10 and 15 minutes and unfrozen controls were planted in steamed soil infested with *Fusaria* or bacteria isolated from typical phloem-injury lesions. For each organism 1 pot was used with each lot of plants, or a total of 24 pots containing 120 plants.

(3) Plants frozen for 15 minutes and unfrozen controls were planted in steamed soils infested with *Fusaria* or bacteria as above, but in addition some of the inoculum was inserted in a wound in the taproot just below the crown of each plant. For each organism 1 pot was used with each lot of plants, or a total of 16 pots containing 80 plants.

(4) Plants frozen for 10 and 15 minutes and unfrozen controls were planted in soil not steamed and not infested. For each lot of plants 4 pots were used, or a total of 12 pots containing 60 plants.

In the experiment there were used 320 plants, of which 80 were frozen for 10 minutes, 120 were frozen for 15 minutes, and 120 were unfrozen. Likewise 120 plants were left uninoculated, and 200 were inoculated in wounds or planted in infested soil.

The organisms used consisted of five species of *Fusarium* and three species of bacteria. These were chosen as being representative of the organisms most commonly isolated. The *Fusaria* were grown on sterilized oats in flasks and the bacteria on nutrient agar. As some of the oats as well as the fungus mycelium was added to the soil infested, a large amount of inoculum was present in all cases.

The experiment was initiated on April 6, 1928, and notes were taken on the frozen plants on April 27 and on the unfrozen controls on May 16. Although many of the frozen plants were so severely injured that they died, some grew fairly well. In no case did any typical phloem injury or heart rot such as has been described and illustrated develop. Neither was there any evidence that the organisms used in the inoculations were capable of attacking unfrozen plants. Some of the frozen roots had decayed somewhat, especially at the lower ends, and in a few cases the *Fusarium* placed in the soil was reisolated from the decaying plant. However, here again no one organism could be consistently isolated from these roots. The plants in the steamed uninfested soil as well as in the unsteamed soil contained *Fusaria* and bacteria.

This experiment was duplicated, using twice as many smaller plants from the field. However, the frozen plants were so severely injured that many of them did not recover. In this experiment, as in the previous one, unfrozen control plants failed to show any evidence of infection.

The results of these experiments indicate that the microorganisms associated with phloem injury and heart rot are not parasitic on the unfrozen plants. Apparently these organisms are capable of living in badly frozen roots and perhaps can aid in their ultimate disintegration. Just how much progress such fungi and bacteria can make in moderately injured tissue has not been determined.

Typical phloem injury and heart rot have been found in plants in the field early in the spring when the temperature was still so low that it seems probable that the microorganisms in the soil had not yet

become very active. On the other hand, these troubles are more commonly found in the field later in the season after ample opportunity has been afforded for the action of microorganisms. This does not mean, however, that microorganisms are a factor in the development of these typical lesions. The collapse of the dead and injured cells is probably sufficient to produce the type of lesions so commonly found in frozen alfalfa plants.

HEALING OF OPENINGS PRODUCED IN RAYS BY FREEZING

It has been pointed out that splitting of the rays of alfalfa roots is a very common result of freezing and that in some instances most if not all of a given lot of plants may show such splitting. The amount of ray splitting in plants receiving the same treatment varies considerably. More or less splitting may occur unaccompanied by any evident injury. Frequently, however, many of the cells adjacent to the cracks are injured or even killed outright. Splitting alone probably would not usually be fatal to the plant, but splitting is so frequently accompanied by the killing of cells that it is hard to consider one without the other. In the present discussion, however, splitting is emphasized, since the presence of cracks and their healing were the chief points in mind when these studies were made.

It was observed by F. R. Jones that the roots of alfalfa plants collected during the winter and early summer frequently showed evidence of ray splitting, while many of those obtained from the same field later in the season did not. This same thing was observed subsequently by the writer while studying sections of varieties and strains of alfalfa to determine the comparative amount of ray splitting. It was apparent that many of these openings in the rays must be closed either by mechanical pressure, by the formation of new cells, by the enlargement of neighboring cells, or perhaps by a combination of all of these methods.

Portions of the taproots about 0.5 cm. in length taken about 2 cm. below the crown of 2 to 5 plants of each of 16 varieties of alfalfa were collected on January 31, April 16, June 1, June 27, and August 1, 1928. These specimens were placed at once in a fixer, infiltrated with paraffin, sectioned, and stained. The method of staining was similar to that described by Jones (2). The varieties or strains of alfalfa used were as follows: Grimm, Cossack, Hardigan, Kansas common, Turkestan, Lebeau, Argentine, Dakota, Utah, South African, Spanish, Italian, Smooth Peruvian, Hairy Peruvian, Arizona, and India. The plants were growing in rows in a small level plot on a fairly uniform well-drained soil at Manhattan, Kans. All the plants were of the same age, the seed having been sown in April, 1927.

The data accumulated in these studies are given in Table 5. It would have been desirable to use more plants of each group, but they were not available. All the plants of the India variety which survived the winter were used in the first three collections. Although the number of plants of each variety used each time was rather small, a total of nearly 300 plants was studied.

TABLE 5.—Amount of splitting in rays of roots of different varieties and strains of alfalfa collected on different dates in 1928 and the progress of healing of the openings

[In some collections healing is indicated where no splitting was present, this is due to the fact that the cracks were closed during the healing process]

Variety	Jan. 31				Apr. 16				June 1				June 27				Aug. 1			
	Plants with the indicated amount of ray splitting				Plants with the indicated amount of ray splitting				Plants with the indicated amount of ray splitting				Plants with the indicated amount of ray splitting				Plants with the indicated amount of ray splitting			
	Number of plants				Number of plants				Number of plants				Number of plants				Number of plants			
	None	Slight	Moderate	Severe	None	Slight	Moderate	Severe	None	Slight	Moderate	Severe	None	Slight	Moderate	Severe	None	Slight	Moderate	Severe
Grimm.....	4	0	4	0	0	0	5	0	5	0	0	0	4	4	0	0	4	4	0	0
Cossack.....	3	0	2	1	0	0	4	0	2	0	0	4	0	0	0	0	3	3	3	0
Hardigan.....	4	0	0	2	0	0	5	0	3	2	0	0	0	1	1	0	0	0	0	1
Kansas common.....	4	1	1	0	0	0	3	0	1	2	0	0	4	3	1	0	3	3	1	0
Turkestan.....	4	1	3	0	0	0	3	0	1	0	0	0	4	4	0	0	0	0	0	2
Lebeau.....	5	2	3	0	0	0	4	3	1	0	0	0	4	4	0	0	3	3	0	0
Argentina.....	4	0	3	0	1	0	5	0	3	2	0	0	3	3	1	2	4	4	0	0
Dakota.....	4	1	3	0	0	0	5	0	5	0	0	0	4	3	1	0	4	4	0	0
Utah.....	5	0	1	4	0	0	5	0	2	3	0	0	4	4	0	0	4	4	0	0
South African.....	5	0	1	0	3	0	5	0	0	4	0	0	4	3	2	1	4	4	3	1
Spanish.....	5	0	0	0	5	0	5	0	2	0	3	1	2	2	0	0	1	4	0	0
Italian.....	4	0	3	1	0	0	4	0	4	0	0	0	4	3	1	3	4	3	1	2
Smooth Peruvian.....	2	0	0	2	0	0	4	0	2	1	1	0	3	3	1	1	0	3	2	0
Hairy Peruvian.....	4	0	1	0	3	0	5	0	3	0	2	0	4	3	1	0	4	4	3	1
Arizona.....	4	0	0	0	4	0	5	3	0	0	2	2	4	4	0	0	4	4	4	0
India.....	4	0	0	0	4	0	4	0	1	2	1	1	4	4	0	0	4	4	0	0
Total.....	64	5	27	12	20	0	69	7	35	14	13	4	54	17	31	6	0	27	51	39
																	1	1	0	36
																	4	7	7	0
																	3	54	47	3
																	7	0	3	35

The data presented in Table 5 show some interesting comparisons. Expressed in percentages, 92.2 per cent of the plants showed ray splitting in January. The amount of splitting gradually decreased as the season advanced, being 89.8, 68.5, 23.5, and 13, respectively, for April 16, June 1, June 27, and August 1. The percentage of plants showing no evidence of splitting naturally increased as the rifts healed, starting at 7.8 per cent in January and increasing to 10.1, 31.5, 76.5, and 87 per cent on April 16, June 1, June 27, and August 1, respectively. Likewise the percentage of plants showing healing was greater at each collection with the exception of the last, starting at 0 in January and increasing to 5.8, 50, 70.6, and 64.8 per cent, respectively, in the different collections.

Not only did the total number of plants showing rifts in the roots decrease as the season advanced and healing progressed, but the amount of splitting also varied. For example, in the slightly split groups the percentages were 42.2, 50.7, 57.4, 21.6, and 13; in the moderately split ones they were 18.7, 20.3, 11.1, 2, and 0; and in the severely split ones they were 31.2, 18.8, 0, 0, and 0, on January 31, April 16, June 1, June 27, and August 1, respectively.

In Table 6 is given a set of figures derived from those in Table 5. These illustrate clearly the decline from January to August in the percentage of plants showing splitting as well as the increase in the amount of healing. This table also brings out more sharply the contrast between the different varieties in regard to the amount of splitting and the rate of healing. For example, all but four varieties—

Kansas common, Turkestan, Lebeau, and Dakota—showed splitting in 100 per cent of the plants examined in January. In April all but Turkestan, Lebeau, and Arizona showed splitting in 100 per cent of the roots studied. A rather decided change was noticeable, however, on June 1, at which time only four varieties—Grimm, Utah, Hairy Peruvian, and India—showed openings in all of the roots examined, and also on August 1, when only Kansas common, South African, Italian, Smooth Peruvian, and Hairy Peruvian showed any splitting at all.

TABLE 6.—Percentage of roots of different varieties of alfalfa showing ray splitting and healing on different dates in 1928

[Partial summary of figures presented in Table 5]

Variety	Percentage of plants showing ray splitting on—					Percentage of plants showing healing on—				
	Jan. 31	Apr. 16	June 1	June 27	Aug. 1	Jan. 31	Apr. 16	June 1	June 27	Aug. 1
Grimm.....	100	100	100	0	0	0	0	0	50	0
Cossack.....	100	100	50	0	0	0	0	0	100	33.3
Hardigan.....	100	100	33.3	50	0	0	0	0	0	33.3
Kansas common.....	75	100	75	25	25	0	0	75	75	50
Turkestan.....	75	50	50	0	0	0	0	0	0	100
Lebeau.....	60	25	50	0	0	0	0	25	75	33.3
Argentina.....	100	100	66.7	66.7	0	0	0	66.7	66.7	75
Dakota.....	75	100	75	25	0	0	0	75	50	25
Utah.....	100	100	100	0	0	0	0	50	100	75
South African.....	100	100	50	33.3	25	0	0	100	100	100
Spanish.....	100	100	50	0	0	0	20	50	50	100
Italian.....	100	100	75	75	66.7	0	0	100	100	66.7
Smooth Peruvian.....	100	100	50	66.7	50	0	0	100	100	100
Hairy Peruvian.....	100	100	100	25	25	0	0	0	100	100
Arizona.....	100	40	50	0	0	0	40	100	75	75
India.....	100	100	100	-----	-----	0	25	75	-----	-----

The cracks had begun to close in three varieties—Spanish, Arizona, and India—by April 16, and in many others by June 1. In general the healing started first and progressed most rapidly in the least hardy varieties, such as the South African, Spanish, Italian, Smooth Peruvian, Hairy Peruvian, Arizona, and India. On the other hand, Grimm and Hardigan, representatives of the most hardy varieties, showed less evidence of healing than any of the others, although most of the roots of these varieties examined earlier had shown some splitting. The rifts in the more hardy varieties, however, were usually not so large, and there probably was less killing of adjacent cells; consequently many of the openings were closed without leaving any evidence of their having been present except possibly some enlarged ray cells resulting in some cases in a slight widening of the rays themselves. No healing was seen in the Turkestan roots until August 1. Lebeau, followed by Turkestan, appeared to have fewer rifts in the rays than any other variety studies.

Considerable tearing apart of cells of the phloem parenchyma was observed in many of the most severely split roots. This disruption of the cellular structure was often accompanied by the killing of the cells themselves. Rifts in this part of the root usually are comparatively small and are also frequently closed by the formation of new cells.

These histological studies failed to disclose any criterion that might serve to distinguish definitely between hardy and nonhardy varieties

of alfalfa. The investigations, however, were not exhaustive, as they dealt largely with the relative amount of splitting and did not include a detailed study of the proportion of the various kinds of tissues or other histological differences in the roots.

It having been shown that alfalfa roots frozen artificially as well as in the field may be more or less severely split, and that these openings may close up after a period of growth, it is of interest to look more closely at the nature of the healing process. A clear picture of severe splitting produced by artificial freezing as described elsewhere is presented in Figure 6. Microscopic study showed not only that the rays were split badly but also that many of the cells were killed, and it seems probable that the plant would have died or that after a period of growth it would have looked somewhat like that illustrated in Figure 8, B. There is, of course, no evidence of healing in this root, as it was sectioned at once after freezing.

One type of healing is evident in Figure 8, A and B, where a wound periderm has been laid down just under the dead portion of the phloem. Another type of healing is illustrated in Figures 7, B, and 8, A, where there are groups of cells in or adjacent to certain of the cracks (some of which are indicated by the letter *a*) that are larger than any of their neighbors. These, the writer believes, have been formed since the roots were frozen, in response to a wound stimulus. If this interpretation is correct, a considerable amount of healing can take place in a period of 40 days under good growing conditions. Other examples of rifts closed in a similar manner are illustrated in Figures 9 and 10.

A photomicrograph of the center of a cross section of the taproot of an alfalfa plant of the Arizona variety is shown in Figure 9, A. This is from a root included in the June 27 collection listed in Table 5. The black lines consisting of the dead cells adjacent to the rifts indicate where the frost cracks had been. The large parenchymatous cells have filled these openings, and the walls of the injured cells have been crushed together in such a manner that only the black lines are now visible. In some instances, as in Figure 9, A, *a*, and in other roots studied, the openings are not yet closed completely. On the other hand, in roots where healing is well advanced the cracks usually are no longer visible.

A section of another root of the Arizona variety collected on June 1, illustrated in Figure 9, B, has the rifts filled up entirely. This illustration is an enlargement of a portion of the center of the root shown in Figure 10, A and B, presented here to give a clearer picture of the character of the cells which fill in these cracks. These cells appear to be parenchymatous in character and to have originated either from ray cells contiguous to the openings or from parenchyma cells, which are abundant in the center of the root as well as in the wood itself. That there are cells all through these roots which are potentially meristematic in nature is evident by the fact that complete layers of wound periderm are laid down about many types of wounds. For example, a band of wound periderm several cells in thickness surrounds the dead central part of the root illustrated in Figure 10, A, and similar layers of cells are seen just beneath the injured phloem in Figure 8, A and B. Such cells, and especially those nearest the injured tissue, sometimes give a positive reaction for lignin when treated with phloroglucin and hydrochloric acid, and in other cases they give a positive

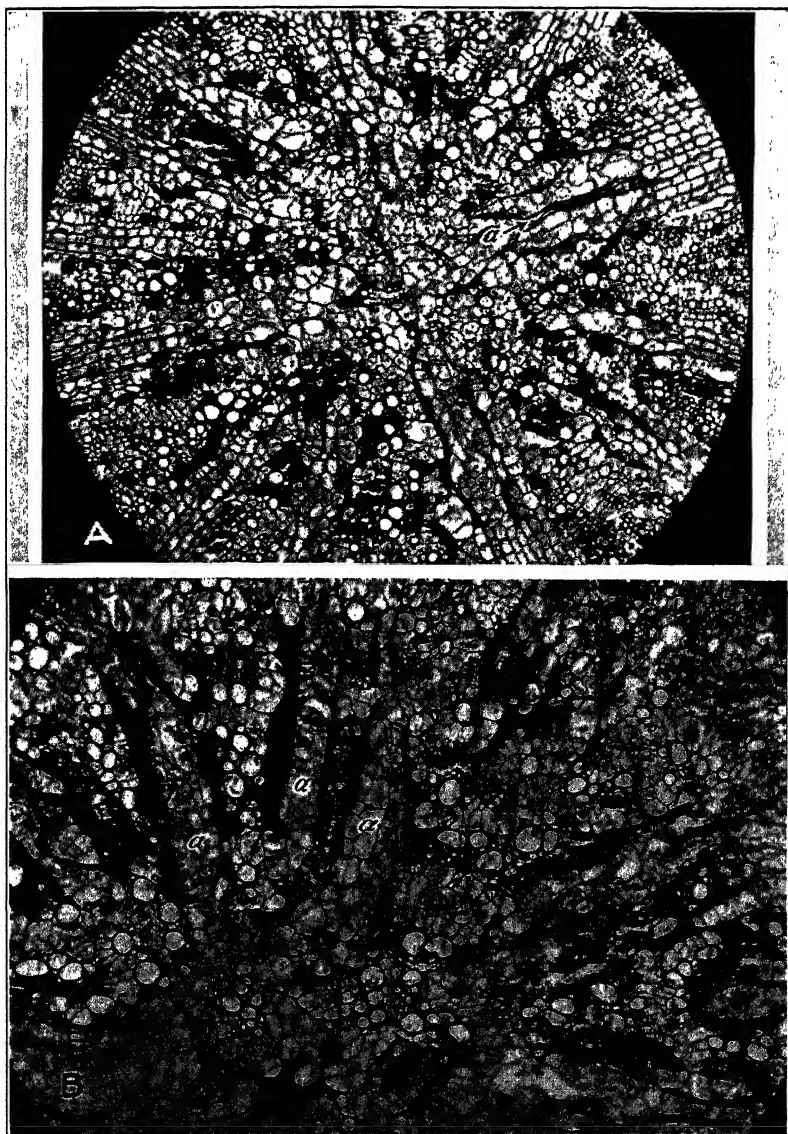


FIGURE 9.—A, Center of a cross section of a taproot of an alfalfa plant of the Arizona variety brought from the field on June 27, 1923. The black lines indicate the position of the frost cracks. The cells killed by the freezing were pressed aside as the open spaces were filled with new cells. One crack not entirely closed is evident in the upper right side of the picture as indicated by *a*. $\times 42$. B, Portions of a section of another root of the same variety collected June 1, 1923. The rifts are completely healed in this root and many new cells have been formed. This is an enlargement of a portion of the section shown in Figure 10, B, presented here to show more clearly the character of the cells that fill in these cracks, *a*. $\times 81$

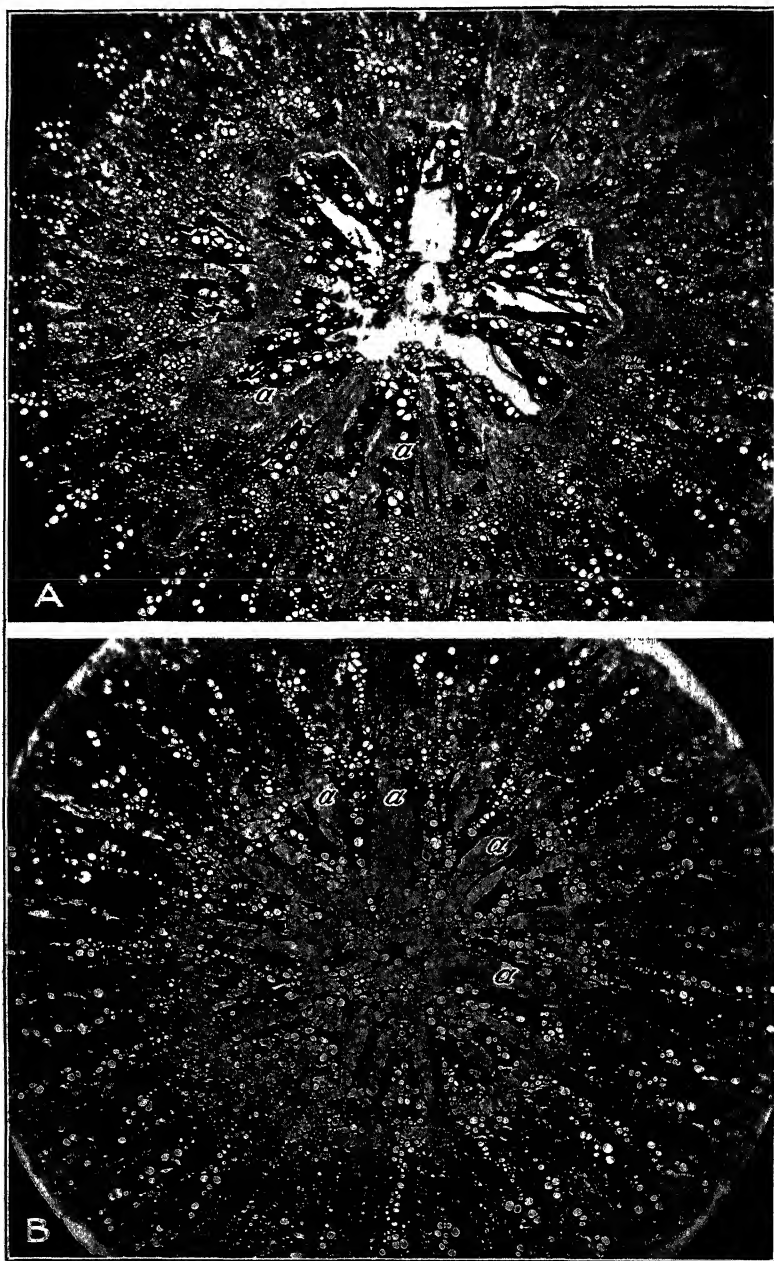


FIGURE 10.—A, Cross section of the center of an alfalfa root of the Arizona variety brought from the field on June 1, 1928. This figure shows a case of typical heart rot. The dead center of the root has been cut off from the live tissue outside by a band of wound periderm *a*. There was killing of cells as well as some splitting of rays outside of the dead central area. $\times 35$. B, Cross section of the same root taken 2 inches below A. There is evidence of much splitting and killing of cells as in A, but the injury was not so severe and the cracks have been filled up completely by the formation of new parenchymatous cells *a*. This section was taken just below the portion of the root showing distinct heart rot. $\times 30$

suberin reaction with Sudan III and are insoluble in 50 per cent chromic acid. They stain green with Planeze III b, indicating the presence of lignin, suberin, or cutin in the walls. The dark lines bordering the healed rifts which have been described as crushed cell walls do not seem to have undergone much, if any, modification, as they stain like the surrounding parenchyma cell walls with all stains used. The large parenchymalike groups of cells which have closed up certain of the rifts have cellulose walls, as indicated by their staining reaction.

Figure 10, A and B, shows sections of the same taproot, but B was taken 2 inches lower than A, which came from near the crown. These sections show that the plant had a typical case of heart rot in the region of the crown, the tissues at the center having been so badly frozen that they could make no recovery. Two inches below, however, although the injury appears to have been quite severe, enough cells survived to initiate new growth, and the cracks were filled completely.

The data presented here seem to justify the following general conclusions: (1) A large percentage of the roots of alfalfa plants growing in climates where the temperature falls below -3°C . or thereabouts for extended periods may show slight to severe splitting in the rays each winter and spring due to freezing; (2) as the season advances, fewer roots show moderate or severe splitting, and even those exhibiting slight rifts decrease as the cracks almost, if not entirely, disappear by the end of the growing season; (3) some slight ray rifts appear to close without leaving any trace of their presence, and others leave distinct and clear-cut evidence that large openings have been entirely closed by the formation of new cells; and (4) large dead areas in either the phloem or the center of the root are cut off from the living tissue beneath by meristematic activity. Much variation in the amount of splitting and the rate of healing even under the same climatic conditions must be expected. These will vary with the age of the roots, the degree of hardening, the hardness of the variety, and other factors. Jones (2) pointed out that the ability of alfalfa roots to heal their injuries is greater the first year than in subsequent years.

SUMMARY

Many alfalfa plants of the Kansas common and Grimm varieties were frozen by different methods and for different lengths of time in order to learn something of the manner in which certain types of root injuries that are so common in the field are produced.

The results of these investigations have confirmed the belief of others as well as of the writer that the cracked and decaying conditions of the crown and of the upper part of the taproot of so many alfalfa plants is due to freezing.

The terms "phloem injury" and "heart rot" are used to designate the injury to those parts of the root lying outside of and inclosed by the cambium, respectively. Most of the plants frozen either died outright or recovered without showing any very striking external evidence of injury to the roots. A certain percentage of the plants, however, did develop the lesions described in this paper as phloem injury and heart rot in one to several weeks after they were frozen.

Both phloem injury and heart rot developed in potted alfalfa plants frozen in soil at average temperatures of -6.6° , -8.2° , and

-11.6° C. for 5, 7, and 4 hours, respectively. Phloem injury alone was apparent macroscopically in plants frozen at -11.6°, -13.8°, -14.5°, -17°, -21°, and -21° C. for 3, 6, 7, 4, 2, and 3 hours, respectively. The plants frozen were grown in the field for about eight months and then transferred to the greenhouse. As they were not frozen at once, they were not in a hardened condition.

Phloem injury developed in plants brought from the field and frozen at -14.6° and -14.9° C. for 1½ and 3 hours, respectively, before being set in soil in the greenhouse. The same type of injury developed in 2 out of 5 plants of the same lot subjected to an average temperature of -14° C. for 7 hours. In this case the plants were set in soil in an 8-inch pot before they were exposed to the low temperature.

Phloem injury, heart rot, or both became evident in 19 out of 275, or 6.9 per cent, of the young alfalfa plants frozen in soil at Madison, Wis., in 40 days or less.

Phloem injury was produced in 3-months-old alfalfa plants by removing them from the soil and subjecting them to average temperatures of -14.2°, -12°, -12°, -12°, -6°, and -11.5° C. for 15, 15, 20, 25, 40, and 10 minutes, respectively.

Attempts to produce the typical phloem injury or heart rot in plants in the field in the summer by covering them with a can containing ice and salt mixture were unsuccessful. The plants could be killed readily by this means, but they either died or recovered without developing the typical winter-injury lesions on the roots.

Histological studies of some of the frozen roots showed that a typical splitting apart of the cells, especially along the rays of the root, usually was present. This tearing apart and the death of the cells in localized areas due to freezing, followed by their complete collapse and decay, result in the formation of the lesions described as phloem injury and heart rot. The typical splitting in artificially as well as in naturally frozen plants is illustrated.

Microorganisms are commonly associated with the typical winter-injury lesions under discussion. The parasitism of five species of *Fusarium* and three of bacteria isolated from typical phloem injury and heart rot was tested. In no case did they infect unfrozen plants. It seems clear that these associated microorganisms are for the most part at least saprophytes or at best very weak parasites.

Histological studies of the roots of nearly 300 plants of 16 different varieties or regional strains of alfalfa have shown that the rays are more or less severely split during the winter. If the injury is not too extreme the cracks formed are practically all closed during the following growing season.

LITERATURE CITED

- (1) HILL, D. D., and SALMON, S. C.
1927. THE RESISTANCE OF CERTAIN VARIETIES OF WINTER WHEAT TO ARTIFICIALLY PRODUCED LOW TEMPERATURES. *Jour. Agr. Research* 35: 933-937.
- (2) JONES, F. R.
1928. WINTER INJURY OF ALFALFA. *Jour. Agr. Research* 37: 189-211, illus.
- (3) RUSSELL, H. L., and MORRISON, F. B.
1923. SCIENCE SERVES WISCONSIN FARMS. *Wis. Agr. Expt. Sta. Bul.* 352, 122 p., illus.

-
- (4) STEINMETZ, F. H.
1926. WINTER HARDINESS IN ALFALFA VARIETIES. Minn. Agr. Expt. Sta. Tech. Bul. 38, 33 p., illus.
- (5) THROCKMORTON, R. I., and SALMON, S. C.
1927. ALFALFA PRODUCTION IN KANSAS. Kans. Agr. Expt. Sta. Bul. 242, 42 p., illus.
- (6) WEIMER, J. L.
1927. OBSERVATIONS ON SOME ALFALFA ROOT TROUBLES. U. S. Dept. Agr. Circ. 425, 10 p., illus.
- (7) WIEGAND, K. M.
1906. THE OCCURRENCE OF ICE IN PLANT TISSUES. Plant World 9: 25-38.

MAGNESIUM AND CALCIUM REQUIREMENTS OF THE TOBACCO CROP¹

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INTRODUCTION

Through the use of improved methods, plant physiologists are now finding that the mineral nutrition of the plant is more complex than has been supposed and that apparently the list of indispensable elements must be considerably enlarged. Moreover, it is becoming apparent that under some conditions certain elements that have long been recognized as essential to normal growth but regarded as of no special significance in practical fertilizer usage must in fact be added to the soil if the best results are to be obtained. On the other hand, there is a definite trend in the fertilizer industry, largely for economic reasons, toward the use of relatively pure chemicals intended to furnish in highly concentrated form and comparatively free from "filler" or supposedly inert material the three elements, nitrogen, phosphorus, and potassium, which have been generally considered as furnishing a complete fertilizer. Mixtures of potassium nitrate and an ammonium phosphate or of ammonium nitrate and a phosphate of potassium are examples of a fertilizer of this sort. Experiments and observations discussed herein seem to indicate, however, that on some of the important tobacco soils the use of such materials alone can not be relied upon to give satisfactory results. The chief difficulty seems to be that the three elements mentioned do not under the circumstances constitute a complete fertilizer rather than that there is any failure of these elements in the forms used to function normally. Apparently lack of magnesium and calcium in the mixture is involved.

In a preliminary paper published in 1923 (*1*)² it was shown that on certain soils, when no magnesium is supplied in the fertilizer or from other sources, a distinctive chlorosis is likely to develop in the leaves of the tobacco plant, due to a breaking down of the chlorophyll. It is the purpose of the present paper to give somewhat more detailed information concerning the magnesium requirements of the tobacco crop and methods for supplying them and to make available the results of preliminary observations on the need of also supplying calcium in the tobacco fertilizer on certain soils. Little in the way of a background for the problems involved can be derived from accumulated experience in fertilizer usage or from the immense amount of ferti-

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² Reference is made by number (italic) to "Literature Cited," p. 168.

lizer experimentation which has been carried out with tobacco and other crops, because the fertilizers and fertilizer materials used almost invariably have been of a more or less complex chemical composition. For example, it is obvious that as long as superphosphate is an important constituent of the fertilizer the plant is not likely to show evidence of an insufficient supply of calcium. Similarly, many organic materials of plant or animal origin which have been widely used as components of the complete fertilizer contain notable quantities of both calcium and magnesium. It should be kept in mind also that continued use of such materials over a period of years tends to set up in some soils a limited and essentially temporary reserve of calcium and magnesium which otherwise would not be present.

MINIMUM MAGNESIUM REQUIREMENTS OF TOBACCO

In the present connection the expression "minimum magnesium requirements" has reference to the minimum content of magnesium in the plant which will serve to prevent the appearance of visible symptoms of magnesium hunger, commonly spoken of as sand drown. The minimum quantities of magnesium required to prevent these symptoms, of course, would not necessarily be sufficient for the maximum growth of the plant.

The symptoms of magnesium hunger in tobacco have been described in the paper mentioned above (1). In other plants the symptoms are more or less similar to those observed in tobacco, but in general the trouble is not so readily diagnosed. In corn the lower blades of the plant show a striped effect, due to the breaking down of the chlorophyll in the areas between the veins. There is a general similarity in the distinctions between magnesium hunger and potassium hunger to those observed in tobacco. In soybeans the leaf presents an appearance clearly suggestive of that seen in tobacco. The tendency of the lower leaves to shed, however, interferes with the full development of deficiency symptoms. Cotton presents very striking symptoms of magnesium deficiency. In the initial stages the bleaching effect in the older leaves, due to decomposition of chlorophyll, may be observed in the margins and in the areas between the larger veins. On the upper surfaces of the leaves, however, development of anthocyanin soon follows the disappearance of the normal green, so that when viewed from above the leaves, with the exception of the principal veins, present a bright red appearance. As far as observed the under surfaces of the leaves do not show the red color.

The plots used in studying the minimum magnesium requirements were located at the cooperative tobacco field station at Upper Marlboro, Md., the Connecticut Tobacco Substation at Windsor, Conn., and the North Carolina Tobacco Branch Station at Oxford, N. C. In a limited number of similar tests on Tifton sandy loam soil at the Georgia Coastal Plain Experiment Station at Tifton, Ga., definite symptoms of magnesium deficiency have not been observed, but further work is needed before final conclusions can be drawn. The plan of procedure has been to ascertain the magnesium content of entire plants and of leaves and stalks of plants showing various degrees of magnesium hunger and grown on soils known to be deficient in magnesium, with and without applications of this element to the soil. Since the symptoms of magnesium deficiency first appear on the lower

leaves of the plant, it is a simple matter to find plants showing definite gradations in these symptoms in the leaves at increasing heights on the stalk. The results of the tests are shown in Table 1.

TABLE 1.—*Magnesium content of tobacco leaves manifesting symptoms of magnesium hunger and of comparable leaves free from these symptoms*

[At Upper Marlboro the leaf samples were taken in the third year of the test]

Locality	Plot No.	Leaf sample No.	Magnesium applied each year		Composition of leaf		Visible symptoms of magnesium hunger
			Pounds per acre	Source	Mg	MgO	
					<i>Per cent</i>	<i>Per cent</i>	
Upper Marlboro, Md.	13	2	0	-----	0.15	0.25	Well defined.
	13	3	0	-----	.22	.37	None.
	14	5	3	Cottonseed meal	.12	.20	Moderate.
	14	6	3	do.	.18	.30	None.
	12	4	120	Dolomitic limestone	.35	.58	Do.
	6-D	13	0	-----	.10	.17	Severe.
	6-B	9	120	Dolomitic limestone	.65	1.08	None.
	3	1-a	110	-----	.08	.13	Severe.
	11	2-a	24	Magnesium sulphate	.42	.70	None.
	7	21	0	-----	.16	.27	Well defined.
Windsor, Conn.	7	22	0	-----	.84	.56	None.
Oxford, N. C.	6	31	0	-----	.20	.33	Moderate.
	7	32	0	-----	.27	.45	None.

The tests at Upper Marlboro, Md., were located on Collington soil, ranging from the sandy-loam to the loamy-sand type. Plots 3, 11, 12, 13, and 14 received as fertilizer 40 pounds of nitrogen, 64 pounds of phosphoric acid, and 80 pounds of potash per acre. The phosphoric acid was derived from dicalcic phosphate and the potash from high-grade sulphate. On plot 14 the nitrogen was derived from cottonseed meal, while on the other plots nitrate of soda was used. In addition to the above-mentioned materials, plots 12 and 13 received 1,000 pounds of dolomitic limestone (12 per cent magnesium) and calcitic limestone, respectively, per acre, applied broadcast, and plot 11 received 250 pounds of Epsom salt per acre, applied in the drill. Sample 2 from plot 13 (Table 1) consisted of lower leaves from plants definitely showing magnesium hunger, while sample 3 contained leaves situated immediately above those of sample 2 on the same plants. While there was no evidence of magnesium hunger in sample 3, it is clear that the magnesium supply of these leaves must have been near the minimum requirements for preventing the disease. The same relations apply with respect to samples 5 and 6 from plot 14. Sample 4 from plot 12 was taken from the same position on the plant as samples 2 and 5. Samples 1-a from plot 3 and 2-a from plot 11 likewise are comparable with samples 2 and 5.

Plots 6-b and 6-d (Table 1) received uniformly as fertilizer on an acre basis 30 pounds of nitrogen in the form of dried blood, 48 pounds of phosphoric acid in the form of dicalcic phosphate, and 73 pounds of potash in the form of high-grade muriate. Plot 6-b also received 1,000 pounds of dolomitic limestone per acre. Samples 13 and 9 consisted of lower leaves comparable to similar samples mentioned in the preceding paragraph.

Plot 7 at Windsor, Conn., received as fertilizer on an acre basis 40 pounds of nitrogen derived from dried blood and nitrate of potash,

64 pounds of phosphoric acid derived from dicalcic phosphate, and 80 pounds of potash derived from nitrate of potash. The dicalcic phosphate also supplied 35 pounds of calcium per acre. Three weeks after transplanting, an additional 40 pounds of nitrogen per acre was applied in the form of nitrate of soda. The soil employed in the test is a light sandy loam of the Merrimac series. On one end of the plot, represented by sample 21, decided evidence of magnesium deficiency appeared, while on the other end, represented by sample 22, there was no indication of the disease. The samples consisted of all leaves on the cured plants.

At Oxford, N. C., plots 6 and 7 received uniformly as fertilizer on an acre basis 32 pounds of nitrogen derived from dried blood, 64 pounds of phosphoric acid (and 35 pounds of calcium) derived from dicalcic phosphate, and 36 pounds of potash, the potash being derived from the sulphate in case of plot 6 and from the muriate in case of plot 7. The soil on which the plots were located is the Durham sandy loam. The leaf samples from the plots were composed of all leaves on the cured plants.

The data presented in Table 1 make it possible to determine with a fair degree of accuracy the minimum magnesium content in the leaf of the tobacco plant which will prevent symptoms of magnesium deficiency as evidenced by a premature breaking down of chlorophyll. It appears that the critical point is reached when the content of magnesium falls to approximately 0.2 per cent, which is equivalent to 0.33 per cent magnesia (MgO). When the content falls much below this figure the disease becomes severe. The value of 0.2 per cent magnesium for tobacco showing moderate symptoms at Oxford, N. C., is somewhat too high, since it applies to all the leaves on the plant, whereas only the lower leaves were actually diseased. It is believed that the slightly higher magnesium content in the leaves from plot 7 at Oxford is due to the solvent action of the muriate of potash on the magnesium of the soil. It seems safe to assume that tobacco leaves with a content of not less than 0.25 per cent magnesium or about 0.4 per cent magnesia will not ordinarily show symptoms of deficiency.

According to Willstätter and Stoll (8), the average content of chlorophyll in green leaves is about 0.8 per cent of the total dry weight, and pure chlorophyll contains 2.7 per cent magnesium. On this basis the magnesium contents of the chlorophyll would be less than 0.03 per cent of the dry weight of the leaf. It is evident that in order to prevent breaking down of chlorophyll the total magnesium content of the leaf must be several times the quantity present in the chlorophyll.

In connection with the problem of magnesium deficiency in individual leaves, it is of interest to consider the distribution of this element in the plant as a whole. The results of studies in this direction are shown in Table 2, along with a limited number of observations on other crop plants under similar conditions. The soils employed were the same as for the tests reported in Table 1 except that the soybeans at Oxford, N. C., were grown on Durham coarse sandy loam. The tobacco material was taken mostly from the same plots that were used for the preceding tests, and the fertilizer treatment can be readily ascertained in each case by referring to the plot number in column 2 of Table 1. At Oxford, plot 1 in tobacco received the same fertilizer as plots 6 and 7 except that no potash was applied. At Windsor, Conn., the treatment of plot 1 was the same as that of

plot 7 in Table 1, and plot 5 differed from these only in that 36 pounds of magnesium per acre was applied in the form of Epsom salt. The treatments of corn plots 13 and 12 were identical with those of the tobacco plots of the same numbers. The plots in potatoes and sweetpotatoes received uniformly on an acre basis 40 pounds of nitrogen as dried blood and nitrate of potash, 64 pounds of phosphoric acid and 35 pounds of calcium as dicalcic phosphate, and 80 pounds of potash as nitrate. The soybeans received no fertilizer, but 1,000 pounds of dolomite per acre had been previously applied to plot 2.

TABLE 2.—*Differences in the distribution of magnesium and calcium in the leaves and stalks (and tubers or thickened roots) of tobacco, corn, potatoes, sweetpotatoes, and soybeans when the supply of magnesium in the soil is varied*

Crop and locality	Plot No.	Visible symptoms of magnesium hunger	Percentage of magnesium in—						Percentage of calcium in—							
			Leaves				Stalk	Tubers (or roots)	Whole plant	Leaves				Stalk	Tubers (or roots)	Whole plant
			Lower	Middle	Upper	Average				Lower	Upper	Average	Stalk			
Tobacco: Upper Marlboro, Md.	6D	Severe	0.10	0.13	0.21	0.13	0.16	0.14	—	—	—	—	—	—	—	—
	6B	None65	.41	.41	.51	.28	.43	—	—	—	—	—	—	—	—
	13	Well defined	—	—	—	.16	.20	.17	—	—	—	—	—	—	—	—
	14	Moderate	—	—	—	.18	.24	.20	—	—	—	—	—	—	—	—
	12	None	—	—	—	.41	.31	.38	—	—	—	—	—	—	—	—
Oxford, N. C.	6	Moderate	—	—	—	.20	.14	.18	—	—	3.46	0.84	—	2.59	—	—
	7	None	—	—	—	.27	.11	.22	—	—	3.67	.82	—	2.72	—	—
	1	do.	—	—	—	.37	.16	.30	—	—	4.60	1.08	—	3.43	—	—
Windsor, Conn.	1	Well defined	—	—	—	.18	.15	.17	—	—	—	—	—	—	—	—
	5	None	—	—	—	1.24	.27	.92	—	—	—	—	—	—	—	—
Corn: Upper Marlboro, Md.	13	Well defined13	—	.13	.13	.20	.18	1.09	0.71	.87	.44	—	.57	—	—
	12	None35	—	.23	.29	.29	.29	.92	.61	.77	.31	—	.44	—	—
Potatoes: Upper Marlboro, Md.	1	Doubtful	—	—	—	.22	0.12	.18	—	—	1.51	—	0.12	.92	—	—
	3	None	—	—	—	.55	.13	.34	—	—	1.08	—	.07	.58	—	—
Sweetpotatoes: Upper Marlboro, Md.	1	Mild	—	—	—	.40	—	.08	.13	—	2.86	—	.20	.71	—	—
	3	None	—	—	—	.71	—	.06	.20	—	2.59	—	.21	.71	—	—
Soybeans: Oxford, N. C.	1	Moderate	—	—	—	—	—	.30	—	—	—	—	—	—	—	—
	2	None	—	—	—	—	—	.59	—	—	—	—	—	—	—	—

The results show that when there is definite evidence of magnesium deficiency the upper leaves of the tobacco plant contain considerably more of this element than the lower ones and that the average content of the leaves as a whole does not differ much from that of the stalk. The stalk, however, usually contains slightly more magnesium than the leaves. With a more liberal supply of magnesium available to the plant, the lower leaves contain considerably more than the upper leaves and the leaves as a whole contain decidedly more than the stalk. It appears that symptoms of deficiency may be expected when the content of magnesium in the plant as a whole falls to 0.21 per cent or thereabouts.

Symptoms of magnesium deficiency may occur in young tobacco plants in the seed bed. In one instance in Granville County, N. C., seedlings showing distinct evidence of magnesium hunger were found to contain 0.13 per cent magnesium as compared with 1.48 per cent calcium.

The limited data available indicate that under similar conditions the minimum magnesium requirements of tobacco and corn are about the same so far as shown by the magnesium content of the plant, and about the same relations seem to hold in the distribution between leaf and stalk. With the same conditions as to soil and fertilization which produced magnesium deficiency in tobacco, no distinctive symptoms could be recognized with certainty in potatoes, but in sweetpotatoes a mild though distinct bleaching of the green leaves was seen. The figures shown in Table 2 for average content of magnesium in the leaves of potatoes and sweetpotatoes refer to samples also containing the aerial stems. It is evident that addition of magnesium to the soil increased the content of this element in the potatoes, sweetpotatoes, and soybeans.

PHYSIOLOGICAL AND BIOCHEMICAL RELATIONS IN MAGNESIUM DEFICIENCY

In the earlier work on magnesium-deficiency symptoms (sand grown) in tobacco (1), certain biochemical observations were made, but the material employed was taken from plots that were not strictly comparable with respect to magnesium relations, since sulphate of potash was used in one case and muriate of potash in another. It was difficult, therefore, to distinguish with certainty between effects due to magnesium and those due to the sulphate and chlorine ions. Accordingly, additional and more complete studies have been carried out under conditions that were more strictly comparable. Samples of full-grown leaves were taken from a position immediately below the center of the plant, the green weights were ascertained, and the leaf areas were determined by tracing on paper. The leaf tissue was killed by exposure to chloroform vapor, and the material was then quickly dried in the oven. The fertilizer treatments of the plots at Upper Marlboro, Md., on which the plants were grown have been described (p. 147), except that of plot 10, not previously mentioned, which received the same treatment as plot 11 save that the potash and the magnesium were derived from the chlorides instead of the sulphates. The leaves from plots 3 and 13 showed definite symptoms of magnesium hunger. The 1924 samples contained 12 leaves, and the 1925 samples contained 30 leaves. The area, green and dry weights, and water content of the leaves are shown in Table 3.

TABLE 3.—*Effect of magnesium deficiency on green and dry weights, area, and water content of mature tobacco leaves at Upper Marlboro, Md.*

Year	Plot No.	Visible symptoms of magnesium hunger	Total leaf area	Total weight		Moisture content	Weight of leaf per square foot		Weight of water per square foot of leaf
				Green	Dry		Green	Dry	
			Sq. feet	Gm.	Gm.	Per cent	Gm.	Gm.	Gm.
1923	12	None	17.53	418.1	80.3	80.8	23.85	4.583	19.27
	13	Well defined	15.48	418.6	60.6	85.5	27.04	3.915	23.12
	8	Severe	22.56	920.6	139.3	84.9	40.82	6.174	34.64
1924	10	None	30.00	1,103.0	196.5	82.2	36.77	6.550	30.22
	11	do	28.99	969.0	182.1	81.2	35.90	6.746	29.15
	12	do	20.15	706.0	151.7	78.5	35.04	7.528	27.50
	13	Severe	17.96	676.7	119.5	82.3	37.68	6.653	31.01

It is evident that the leaves with symptoms of magnesium deficiency (plots 3 and 13) in all cases had only a slight reduction in total green weight but a decided decrease in total dry weight and, of course, a corresponding increase in water content, and there was considerable reduction in total leaf area. On the basis of equal area of leaf, the green weight appreciably exceeded that of the normal leaf, but the dry weight was considerably less. The marked increase in water content when magnesium was deficient possibly was due to the failure of the stomata to function normally when the chlorophyll pigments had become disorganized. The material on which Table 3 is based was subjected to chemical analysis, and the results are shown in Table 4.

TABLE 4.—Effect of magnesium deficiency on the composition of mature tobacco leaves at Upper Marlboro, Md.

PERCENTAGE COMPOSITION OF DRY MATERIAL

Year	Plot No.	Carbohydrates			Organic acids				Protein	Ash		Calcium	Magnesium
		Starch	Reducing sugars (as invert)	Invert sugars (as sucrose)	Citric	Malic	Oxalic	Total		Total	Pure ^a		
1923	12	27.93	4.12	0.21	-----	-----	-----	-----	8.94	-----	-----	-----	-----
	13	18.55	4.54	.23	-----	-----	-----	-----	10.06	-----	-----	-----	-----
	3	18.00	7.82	.16	0.76	4.09	1.23	6.08	11.56	14.26	9.70	1.72	0.08
	10	28.74	7.13	-----	.50	1.92	1.05	3.47	11.50	9.67	7.59	1.37	.39
1924	11	27.53	6.89	.04	.90	3.13	.96	4.99	11.43	10.17	7.61	1.36	.42
	12	29.53	5.38	.11	1.02	2.90	.91	4.83	8.88	9.67	6.96	1.53	.30
	13	20.44	6.12	.11	.80	4.25	1.30	6.35	9.19	15.07	9.52	2.18	.04

WEIGHT IN GRAMS PER SQUARE FOOT OF LEAF AREA, ON DRY BASIS

Year	Plot No.	Starch	Reducing sugars (as invert)	Invert sugars (as sucrose)	Citric	Malic	Oxalic	Total	Protein	Ash	Pure ^a	Calcium	Magnesium
		Starch	Reducing sugars (as invert)	Invert sugars (as sucrose)	Citric	Malic	Oxalic	Total	Protein	Ash	Pure ^a	Calcium	Magnesium
1923	12	1.22	0.19	0.01	-----	-----	-----	-----	0.44	-----	-----	-----	-----
	13	.73	.18	.01	-----	-----	-----	-----	.81	-----	-----	-----	-----
	3	1.11	.48	-----	0.05	0.25	0.08	0.38	.69	0.88	0.60	0.11	0.005
	10	1.88	.47	-----	.03	.13	.07	.23	.75	.63	.50	.09	.03
1924	11	1.94	.48	-----	.06	.22	.07	.35	.81	.72	.54	.10	.03
	12	2.22	.41	.01	.08	.22	.07	.37	.69	.73	.52	.12	.02
	13	1.36	.41	.01	.05	.28	.09	.42	.63	1.00	.63	.15	.003

^a Ash free from carbon, carbon dioxide, and silica.

Since the distinctive pathological feature of magnesium deficiency is in the first instance disorganization of the chlorophyll pigments, it is to be expected that assimilation processes would be first affected. This is clearly indicated in the reduction of the starch reserve in the leaves from plots 3 and 13. Marked accumulation of starch is one of the distinctive features of normal maturation in the tobacco leaf, as is shown by the leaves from the other plots. There are no marked differences in sugar content, indicating that diastatic activity has not been greatly disturbed. The protein values are not entirely consistent, but they show that even with moderately severe chlorosis there is no serious breaking down of protein. The apparent increase in ash content is largely a complementary relationship due to loss of carbohydrate, but in part also it involves partial replacement of readily mobile magnesium by the less mobile calcium. In association with the latter relationship there seems to be a slight increase in accumulation of organic acids, probably in the form of calcium salts. The chemical picture as a whole fully agrees with field observations in

showing that, except in very severe cases and in advanced stages, magnesium deficiency involves, from a physiological standpoint, little more than interference with assimilation due to the breaking down of chlorophyll. As a natural result, growth processes are retarded, but the affected tissues may resist final breakdown for a prolonged period.

As has been suggested in a preceding paragraph, the chlorine ion apparently increases the availability of the magnesium in the soil, and in the earlier report on magnesium deficiency (1) considerable data were presented bearing on the comparative effects of sulphate and muriate of potash under conditions of restricted magnesium supply. In plots 10 and 11 of the present series, however, the plants received an ample supply of magnesium and the two treatments differed only with respect to chlorine and sulphate ions. The data in Table 3 indicate that, independently of the magnesium supply, the chlorine ion (plot 10) has a definite effect on the water relations and vigor of growth of the tobacco leaf. The physiological effects of chlorine on tobacco will receive more detailed consideration in another publication, and in the present connection it will suffice to point out that this element tends to increase the total green weight of the leaf, due primarily to the increased water content maintained in the tissues. This situation results in a considerable increase in leaf area, and, as noted elsewhere (6), it has been observed to exert a decided protection against drought injury in the form of firing or spotting of the leaf.

MAGNESIUM AS A FERTILIZER FOR TOBACCO

In farm practice the occurrence and the severity of the symptoms of magnesium hunger on light-sandy and sandy-loam tobacco soils are greatly affected by weather conditions, particularly the amount and distribution of the rainfall. In comparatively wet seasons evidence of this condition is widespread. It was especially prevalent, for example, in the South Atlantic States and in the Connecticut Valley during the wet summer of 1928. Since attention was first directed to this nutritional disease considerable quantities of dolomitic limestone have been used as a preventive in some sections, and some attention also has been given to the inclusion of magnesium in the tobacco fertilizer. It has been thoroughly demonstrated that application to the soil of comparatively small quantities of suitable forms of magnesium provides an effective preventive of this trouble, but there is as yet little definite information as to the particular types of soil which are likely to be deficient in magnesium. The data presented in the preceding paragraphs make it clear that the particular soils used in these tests are deficient in available magnesium, and it seemed desirable to ascertain the content of these soils in magnesium soluble in hot acid. Analysis was made of representative samples of the soil and subsoil from the plots, or from land adjoining them, at Upper Marlboro, Md., and Windsor, Conn. At Oxford, N. C., the soil samples used for analysis were taken from plots located on Durham coarse sandy loam, and represent the soil on which the data for soybeans (Table 2) were obtained. In this instance the subsoil was not studied. In each instance separate analyses were made of three different samples, but in no case was there any important difference in the results. The average values obtained and the corresponding values for lime content are shown in Table 5.

TABLE 5.—*Magnesium and calcium content (water-free basis) of soils used in plot tests to determine the minimum magnesium requirements of tobacco*

Locality	Type of soil	Magnesium content	Calcium content
		<i>Per cent</i>	<i>Per cent</i>
Windsor, Conn.-----	(Merrimac sandy loam, soil-----	0.24	0.32
	(Merrimac sandy loam, subsoil-----	.27	.35
Upper Marlboro, Md.*-----	(Collington loamy sand, soil-----	.10	.07
	(Collington loamy sand, subsoil-----	.21	.07
Oxford, N. C.-----	Durham coarse sandy loam, soil-----	.024	.09

* Separate samples of soil were used for the magnesium and the calcium determinations.

These results are especially interesting as indicating the wide range in content of magnesium in soils under which deficiency symptoms may occur. These symptoms have been but little less prominent on the Windsor than on the Oxford soil, although the former contains 10 times as much magnesium soluble in strong acid as the latter. On the basis of data given in Tables 1 and 2, a tobacco crop of about 1,200 pounds of dry leaf and 600 pounds of stalks was able to obtain from the Windsor soil only about 3.3 pounds of magnesium, despite the fact that the potential supply in the first 7 inches of the soil is estimated to have been about 6,000 pounds. Obviously, the availability of the magnesium is a very important factor. In Europe, Joulie has often been quoted (3) as holding that productive soils should contain a minimum of 0.18 per cent magnesium (0.30 MgO), but it is apparent from the results on the Windsor soil that a content of 0.24 per cent is not always adequate, at least in the case of tobacco. On the other hand, it is undoubtedly true that magnesium hunger in tobacco will not always occur on soils containing considerably smaller quantities of magnesium. The relative availability of the magnesium and the prevailing weather conditions seem to be factors of major importance.

Numerous chemical analyses of soil types commonly employed in growing tobacco have been reported, but many of these do not include determination of the magnesium content. So far as this value has been given, a large proportion of the figures for the sandy, sandy-loam, and fine sandy-loam types of soil commonly used in growing the flue-cured, Maryland, and cigar wrapper and binder leaf types of tobacco run from 0.1 per cent down to mere traces of magnesium. It should not be surprising that symptoms of magnesium hunger are frequently seen on many of these soils. The heavier loams and silt loams on which dark air-cured and fire-cured, Burley, and cigar-filler and binder types of tobacco are grown apparently contain, as a rule, an ample supply of magnesium for the requirements of the crop. A limited number of experiments conducted on these soils seem to support the analytical data on this point.

In contrast with the variable and uncertain availability of the natural magnesium content of the soil stands the effectiveness and certainty with which applications of soluble salts of magnesium and dolomitic limestone to the soil prevent any evidence of magnesium hunger in the crop. The effect of adding magnesium sulphate to the soil in increasing the magnesium content of the tobacco crop is

well shown in the experiments at Windsor, Conn., some features of which have been given in the preceding tables. The results of these experiments are presented in part in Table 6.

TABLE 6.—*Effect of adding magnesium sulphate to the soil on the content of magnesium in the tobacco crop at Windsor, Conn.*

Plot No.	Magnesium applied per acre	Magnesium content of—			Magnesium ^a removed in crop
		Leaf	Stalk	Whole plant	
	Pounds	Per cent	Per cent	Per cent	Pounds
1.....	0	0.18	0.15	0.17	3.3
3.....	18	.84	.22	.63	14.1
5.....	36	1.24	.27	.92	19.9

^a Data based on analyses here shown and actual plot yields of leaf.

The addition of only 18 pounds of water-soluble magnesium (30 pounds MgO) per acre (in the drill) increased the magnesium content of the leaf four times, and the addition of 36 pounds raised the content to seven times that of the leaf in the control planting. The effect on the magnesium content of the stalk was relatively small. The crop as a whole was able to recover much more than half of the 18 pounds of magnesium added to the soil and about half of the 36 pounds applied. In experiments elsewhere the recovery of soluble magnesium applied as fertilizer, although not always so large, has been quite satisfactory without exception. Over a period of several years the additions of magnesium in the Windsor tests have given moderate increases in yield, but the greater effect has been on the quality of the cured leaf.³ So far as known at present 18 pounds of water-soluble magnesium per acre applied as a fertilizer is ample for preventing symptoms of magnesium hunger or sand drown, and in most cases half of this quantity probably will be effective. It may well be, of course, that larger quantities of magnesium are needed for the production of maximum yield and quality in tobacco than are required to suppress deficiency symptoms. Some data on this point are presented on page 163. Sulphate of potash-magnesia, which contains 5 to 7 per cent magnesium, and sulphate of magnesia appear to be effective sources of water-soluble magnesium for tobacco.

Dolomitic limestone has given uniformly good results as a remedy for magnesium hunger when applied either broadcast or in the drill. Larger quantities of magnesium in this form are required, of course, than when water-soluble salts are used. The detailed results of extensive experiments over a period of years at Oxford, N. C., on the value of dolomitic limestone for the tobacco crop have been reported elsewhere (5, 6). These results show highly profitable returns, and results from somewhat similar tests at Upper Marlboro, Md., which have not yet been published, also show decided benefit from the use of this material. In these experiments the usual practice has been to apply 1,000 pounds of the limestone per acre in the drill or a ton broadcast, although in some instances 500 pounds in the drill has

³ Credit is due H. F. Murwin, agent, of the Office of Tobacco and Plant Nutrition, for having secured data on yield and quality of the crop under the several treatments. For further details of these experiments see Murwin (7).

proved to be sufficient. In some experiments the limestone has been applied each year, while in others it has been applied broadcast once every three years. The results as a whole seem to indicate that 100 to 200 pounds of actual magnesium per acre in the form of limestone will effectively control deficiency symptoms. In the tests at Oxford, N. C., a ton of dolomitic limestone (12 per cent magnesium) per acre applied broadcast once in every three years has been effective.

Experience has shown that the magnesium contained in organic materials of plant and animal origin, such as cottonseed meal, tobacco stems, and barnyard manure, may effectively prevent deficiency symptoms in tobacco when sufficient quantities of the materials are applied to the soil. This is probably one reason why cottonseed meal, which usually contains about 0.6 per cent magnesium, has been so popular in some sections as a constituent of the tobacco fertilizer. Castor pomace also contains about 0.5 per cent magnesium. The data in Tables 1, 2, and 4 showing the magnesium content of tobacco as affected by the available supply in the soil illustrate the variation to be expected in the content of magnesium in crop residues grown on various soils and with various systems of fertilizing. Because of these variations in soil and in fertilizer practice precise data as to the fertilizer value of crop residues with respect to magnesium content can not be given. It appears that the straws of wheat, oats, rye, and barley, as well as grass hay, usually contain 0.1 to 0.15 per cent magnesium, while corn stover ordinarily ranges from 0.15 to 0.3 per cent, and clover hay from 0.3 to 0.5 per cent. For barnyard manure with 75 per cent water, a large number of analyses indicate an average magnesium content of 0.09 to 0.12 per cent, as compared with 0.35 to 0.65 per cent calcium, 0.4 to 0.6 per cent potash, and 0.15 to 0.35 per cent phosphoric acid. The few data available for products of animal origin indicate a magnesium content of 0.15 to 0.3 per cent in tankage and dried ground fish, while dried blood seems to be almost free from magnesium. Wood ashes, which have been extensively used as a fertilizer for tobacco in some sections, usually contain on a dry basis 2 to 3 per cent magnesium. In the fertilizer experiments at Oxford, N. C., basic slag and bone meal have tended to prevent symptoms of magnesium hunger in tobacco, but the quantity of magnesium in these materials was not determined. Rock phosphate and apatite, and therefore superphosphate, sometimes contain small percentages of magnesium.

SYMPTOMS OF CALCIUM DEFICIENCY IN TOBACCO

As previously stated, when calcium is not present in sufficient quantity in the culture medium magnesium may be highly toxic to plants. For this reason it is somewhat difficult to distinguish satisfactorily between symptoms of magnesium toxicity and symptoms of actual calcium deficiency. Since the present discussion of the calcium requirements of tobacco is only preliminary, no attempt will be made to review the literature on the functions of this element in plant nutrition and on the deficiency symptoms developed in water cultures when it is not present in sufficient quantity. The main purpose is to direct attention to the fact that definite calcium-deficiency symptoms may develop in tobacco grown on certain soils unless this element is supplied in proper quantity in the fertilizer or by other means. Evidence

of this fact was first brought to light in connection with a series of plot tests with nitrogen carried out on the Collington loamy-sand type of soil at Upper Marlboro, Md., in collaboration with D. E. Brown, of the Office of Tobacco and Plant Nutrition, the detailed results of which will be presented elsewhere. In these tests provision was made for including commercial Ammo-phos containing 11.5 per cent nitrogen and 45 per cent phosphoric acid. This material was used at two rates in conjunction with sulphate of potash, to supply 20 and 40 pounds nitrogen, 80 and 160 pounds phosphoric acid, and 40 pounds potash per acre. The yields obtained as compared with those produced by equivalent quantities of ammonium sulphate and urea were very poor, and, in fact, were but little better than where no nitrogen at all was used. Evidences of magnesium deficiency having appeared in certain other plots in this series of nitrogen tests, after two years dolomitic limestone containing 12 per cent magnesium was applied uniformly on all plots at the rate of a ton per acre. For a period of six years since the addition of the limestone the Ammo-phos has given substantially as good yields as the other forms of nitrogen.

Subsequently, on the same soil type a test was begun with a mixture of almost pure ammonium phosphate and nitrate of potash furnishing 40 pounds nitrogen, 60 pounds phosphoric acid, and 90 pounds potash per acre (plot A). This mixture was compared with a fertilizer supplying the same quantities of nitrogen, phosphoric acid, and potash in which the nitrogen was derived in equal proportions from cottonseed meal and nitrate of soda, the phosphoric acid from superphosphate, and the potash from the sulphate (plot B). As to the outcome of the test, it will suffice to state that after the first year very poor results were obtained with the ammonium phosphate-nitrate of potash mixture. A large proportion of the plants, in fact, were but little larger at the end of the growing season than when first set in the field. Unfavorable results also have been obtained with this mixture on other sandy and sandy loam soils. Here, as in the Ammo-phos test, however, the addition of dolomitic limestone to the soil largely, if not entirely, corrected the trouble.

In the above-mentioned tests the outstanding feature of the poor results has been the failure of the plants to grow, although distinct evidence of chlorosis in the leaves also has been observed. Additional field experiments, involving the use of pure chemicals, together with pot tests, were next undertaken to reach an explanation of the favorable action of dolomitic limestone in bringing about normal response by the plant to Ammo-phos and the combination of ammonium phosphate and nitrate of potash. In a plot test with a combination of ammonium nitrate, monoammonium phosphate and ammonium sulphate (plot 1) furnishing 40 pounds of nitrogen and 64 pounds of phosphoric acid per acre but no potash, magnesium, or calcium, there was poor growth in the tobacco the first year and almost no growth thereafter.

The most prominent disease symptoms were those of potash hunger. With an addition of 80 pounds of potash per acre in the form of muriate (plot 2) the symptoms of potash hunger disappeared, but still the plants made very little growth, as is shown in Figure 1. When 12 pounds of magnesium per acre in the form of sulphate was added to the fertilizer mixture (plot 3) there was marked improvement in growth of the plants, as is seen in Figure 2. In this case, however, the upper

leaves of the plants showed striking and characteristic abnormalities in development. These abnormalities are well brought out in Figures 3 and 4, which show an individual plant and an individual leaf of a



FIGURE 1.—Plot of tobacco (four rows) which received as fertilizer a mixture of ammonium nitrate, ammonium sulphate, monoammonium phosphate, and muriate of potash in proportions to supply 40 pounds of nitrogen, 64 pounds of phosphoric acid, and 80 pounds of potash per acre. The photograph was made near the end of the growing season. Similar results have been obtained with ammonium phosphate and nitrate of potash. (Compare with Figures 2 and 3.) Scale in feet

plant, respectively, from this plot. The tips and margins of the upper leaves failed to develop normally, and some of the leaves appear as if sections of the laminae had been cut away. There is some mottling,



FIGURE 2.—Plot of tobacco (four rows) which received the same fertilizer as that shown in Figure 1 except 12 pounds of magnesium per acre was added in the form of sulphate. Growth was greatly stimulated, but the upper leaves showed striking abnormalities in development. Scale in feet

but this is not a dominant feature. The lower leaves are normal. This trouble shows little or no resemblance to either potash hunger or magnesium hunger. Similar results were obtained when the ammonium



FIGURE 3.—Individual plant from the plot shown in Figure 2, illustrating abnormalities in leaf development which resulted from a deficient supply of calcium

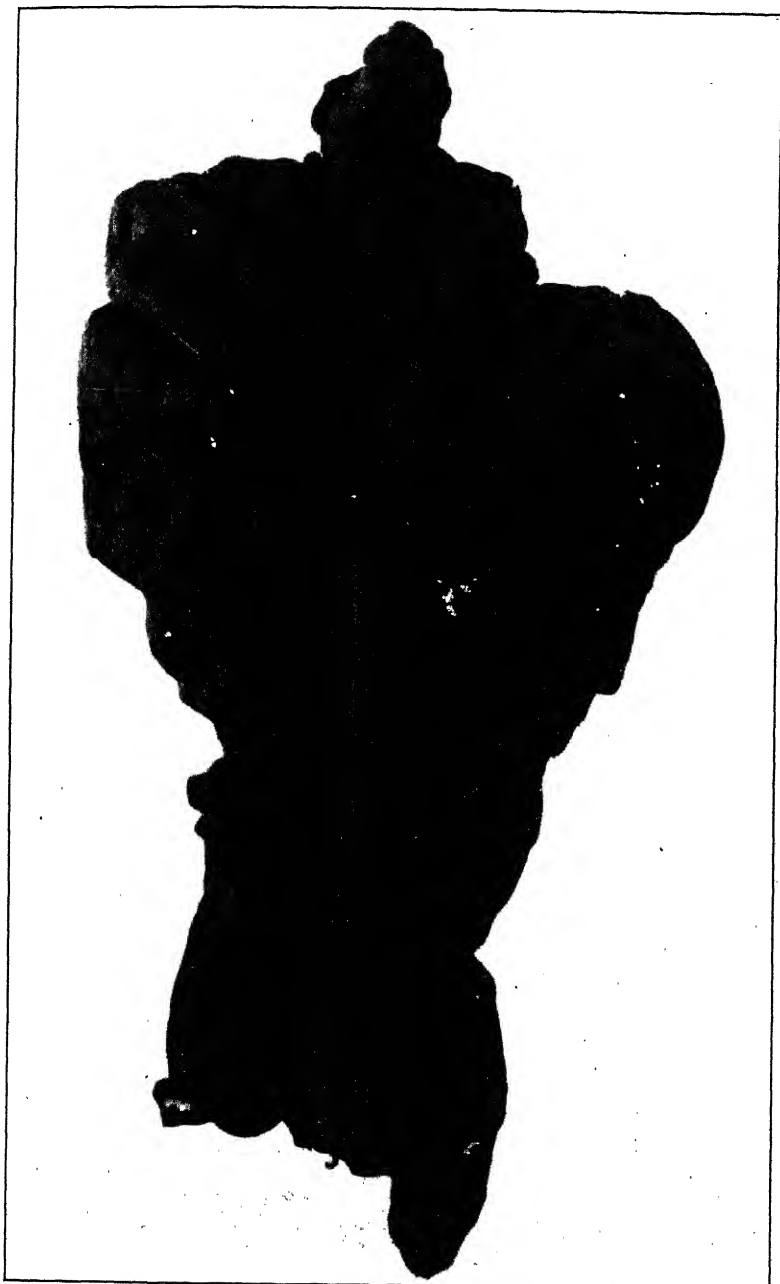


FIGURE 4.—Individual leaf from plot shown in Figure 2, illustrating in greater detail abnormalities in development associated with an inadequate supply of calcium. As shown in Figure 6, the addition of 36 pounds of calcium per acre fully corrected this pathological condition

phosphate was replaced by monopotassium phosphate, with proper reduction in the quantity of muriate of potash applied, so as to keep the total quantity of potash constant (plot 4).

The next step was to supply calcium as a nutrient. This was done by replacing the ammonium phosphate with an equivalent supply of dicalcic phosphate, thus supplying about 36 pounds of calcium per acre. The ammonium nitrate also was replaced with nitrate of soda for a purpose not bearing on the present problem (plot 5). Adding the calcium to the fertilizer mixture had the effect of producing an entirely normal crop of tobacco, as is shown in Figure 5. That the nitrate of soda was not a factor in the improvement obtained is shown by the fact that in an adjoining plot, identical in treatment with plot 3 except that nitrate of soda was substituted for the

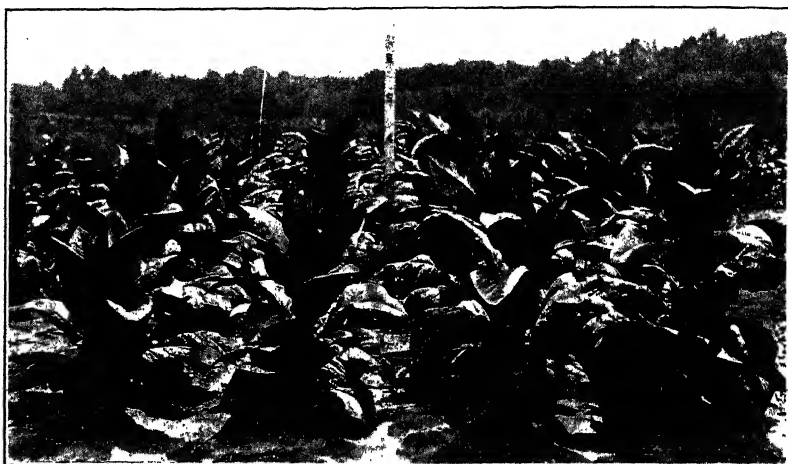


FIGURE 5.—Plot of tobacco which received the same fertilizer as that shown in Figure 2 except that the ammonium phosphate was replaced by dicalcic phosphate and nitrate of soda was substituted for ammonium nitrate. It will be seen that this treatment produced an excellent crop. In numerous supplementary tests ammonium nitrate has given excellent results, and the great improvement in the crop over that in Figure 2 is to be attributed to the calcium supplied in the phosphate. Scale in feet

ammonium nitrate, the tobacco showed the same abnormalities as that in plot 3. Thus, it appears that on the soil in question both magnesium and calcium are essential constituents of the complete fertilizer for tobacco. It should be stated here that this soil is very nearly neutral in reaction, the pH value ranging from 6.5 to 6.8. The interesting question arises whether in these plot tests the striking abnormalities in leaf development which appeared when calcium was not included in the fertilizer were really due specifically to calcium hunger as such or are to be regarded as manifestations of magnesium toxicity in the absence of calcium. These tests in themselves do not permit of a definite answer, but they do show that if magnesium toxicity was involved potash was wholly ineffective as an antagonistic agent.

In this connection it should be recalled that in the ammonium phosphate-nitrate of potash test, where neither magnesium nor calcium was added to the soil, no clearly defined symptoms of calcium deficiency could be seen. This fact suggests that the observed effects

are due, at least in part, to magnesium toxicity. In any event a small quantity of calcium in the fertilizer has proved to be an effective remedy for the trouble. These results in the field were confirmed in pot tests with the same soil. In order to accentuate the effects, the soil was occasionally leached with an excess of dilute nutrient solutions supplying nitrogen, phosphorus, potassium, magnesium, and sulphur, with and without calcium. In the absence of calcium the growing point and upper leaves of the plant invariably showed characteristic pathological effects similar to those seen in the field tests, and only the lower leaves were able to develop normally. The growing point is affected to such an extent that commonly the plant is unable to flower. These tests have shown that magnesium hunger affects primarily the lower leaves of the plant, whereas calcium hunger affects most seriously the upper leaves and embryonic tissues of the growing point. Since the soil in question is deficient in both magnesium and calcium, it is easy to see that anything like normal growth of the plant is impossible when fertilizers are used which supply only nitrogen, phosphoric acid, and potash. When only one of the deficient elements, magnesium or calcium, is added to the fertilizer, growth of the plant is markedly stimulated, but there results a correspondingly marked accentuation of the pathological deficiency symptoms of the element which has not been supplied.

CALCIUM REQUIREMENTS OF TOBACCO

With evidence in hand that under practical field conditions calcium deficiency may occur in some tobacco soils, it was of interest to obtain information as to the minimum content of calcium in the plant which will prevent deficiency symptoms. With this in view, samples of upper and lower leaves of plants were secured from some of the plots at Upper Marlboro, Md., which have been discussed in preceding paragraphs, and the content of magnesium and calcium in the samples was determined. Details of the plot treatments have already been given (p. 156 and 160). In plot A, involving the use of ammonium phosphate and nitrate of potash, the growth of plants on one end (designated A-1) was considerably poorer than that on the other end (designated A-2), and separate samples were taken from the two ends of the plot. The results of the analyses are shown in Table 7.

TABLE 7.—*Calcium and magnesium contents of tobacco showing calcium-deficiency symptoms, and of comparable material free from such symptoms, grown at Upper Marlboro, Md.*

Plot No.	Calcium per acre supplied in fertilizer	Position of leaves	Calcium-deficiency symptoms	Calcium content	Magnesium content
	<i>Pounds</i>			<i>Per cent</i>	<i>Per cent</i>
3.....	0	Upper.....	Pronounced.....	1.00	0.57
3.....	0	Lower.....	Not pronounced.....	1.00	.72
5.....	25	Upper.....	None.....	1.93	.46
5.....	35	Lower.....do.....	2.29	.57
A-1.....	0	Upper.....	Doubtful.....	.94	.26
A-1.....	0	Lower.....do.....	1.30	.26
A-2.....	0	Upper.....	None.....	1.33	.32
A-2.....	0	Lower.....do.....	1.90	.26
B.....	50	Upper.....do.....	1.87	.31
B.....	50	Lower.....do.....	2.43	.23

On plot A no magnesium was supplied in the fertilizer, and on plot B only an insignificant quantity was carried in cottonseed meal used in the fertilizer. In these cases it is apparent that the magnesium content in the lower leaves of the plant is dangerously near the point at which magnesium-deficiency symptoms make their appearance. The magnesium content of the upper leaves equals or exceeds that of the lower ones. The distribution of calcium between upper and lower leaves is decidedly in favor of the latter. This relationship in distribution is in line with the fact that magnesium deficiency first becomes apparent in the lower leaves, whereas ordinarily calcium deficiency is first seen in the upper ones. The marked increase in calcium content in the leaves resulting from addition to the soil of 50 pounds of calcium per acre, seen in comparing treatments B and A, without material change in magnesium content, confirms the conclusion that calcium deficiency was the major cause of poor growth in plot A-1. Furthermore, it appears that with a low magnesium content a calcium content of 1.33 per cent in the upper leaves was sufficient to admit of good growth free from deficiency symptoms, whereas a content of 0.94 per cent was clearly inadequate. In treatments 3 and 5, 12 pounds of magnesium per acre in the form of sulphate was supplied in the fertilizer, and the magnesium content of the tobacco leaves was well above the minimum requirements. In treatment 3, in which the soil received no calcium, the content of this element in the leaves (1 per cent) was sufficient to admit of fair growth, but with development of marked deficiency symptoms. As compared with results on plot A-1, the additional magnesium stimulated growth but accentuated the calcium-deficiency symptoms. On plot 5, as on plot B, a small quantity of calcium in the fertilizer doubled the calcium content of the tobacco leaves and resulted in excellent growth and freedom from deficiency symptoms.

The limited data here presented do not justify sweeping conclusions, but they do show that under the conditions of the tests the minimum calcium requirement of tobacco, as measured by calcium content of the upper leaves, lies between 1.1 and 1.5 per cent (1.5 to 2.1 per cent CaO). The minimum calcium requirement, therefore, is about four or five times the minimum magnesium requirement. It is quite possible that with increased content of magnesium in the leaves the calcium requirement would be materially increased. In the light of published data for other crops it would seem that the calcium requirements of tobacco are relatively high. The data from plots 3 and 5 and those in Table 2 relating to corn and potatoes seem to indicate that magnesium may replace calcium to some extent in the plant when the calcium supply is deficient, but with decidedly harmful results, at least in the case of tobacco.

It seemed desirable to ascertain the potential calcium supply of the soil used in the above-described tests and of the other soils which have proved to be deficient in magnesium. The results are shown in Table 5. It is seen that the Collington loamy sand, which is unable to furnish sufficient calcium for the needs of the crop, contains in both the topsoil and the subsoil 0.07 per cent soluble in hot hydrochloric acid. The calcium content of the Merrimac sandy loam is somewhat greater than the magnesium content. The Durham coarse sandy loam is low in calcium, although the content is appreciably higher than that of magnesium. Whether the Merrimac and

Durham types will produce calcium-deficiency symptoms in tobacco has not been determined. Available data on the calcium content of tobacco soils are somewhat more extensive than those on the magnesium content. Where comparison is possible the average calcium content exceeds considerably the average magnesium content of the sandy and sandy-loam tobacco soils. It is also true, however, that the calcium requirements of the crop considerably exceed the magnesium requirements. Moreover, a comparatively large proportion of the analyses of these soils show a calcium content of less than 0.1 per cent, suggesting that the available supply may not be sufficient for normal crop production. A considerable amount of experimentation will be required before a definite conclusion can be reached as to the extent to which deficiency symptoms and effects are to be expected on these soils when fertilizers free from calcium are used.

BALANCING MAGNESIUM AND CALCIUM IN TOBACCO FERTILIZER

In undertaking to supply magnesium and calcium as nutrients for the tobacco crop in soils found to be deficient in these elements, certain important considerations must be kept in mind, although from a practical standpoint the problems involved are relatively simple. The two most important considerations are: (1) Magnesium may be toxic to the plant unless a sufficient supply of calcium is present; (2) the soil reaction is of considerable significance from the standpoint of disease. The data presented herein, together with the results of pot tests which will not be here considered in detail, show that magnesium may be decidedly toxic even when applied to the soil in the form of finely divided carbonate. Water-soluble magnesium is readily absorbed by the plant, and it has been shown in preceding paragraphs that only 12 pounds per acre applied as sulphate resulted in markedly abnormal growth in tobacco when calcium was deficient in the soil. In the same tests, however, a small quantity of calcium in the form of phosphate was quite effective in preventing this abnormality. In this connection it will be recalled that ordinary superphosphate (acid phosphate) may contain 18 per cent or more of calcium, partly as phosphate and partly as sulphate. Where this material is used at ordinary rates, it would be expected to supply sufficient calcium to prevent deficiency symptoms and also counteract magnesium toxicity unless the magnesia is present in large quantities. An experiment on Durham coarse sandy loam at the Oxford (N. C.) Branch Tobacco Station supports this conclusion. In a test begun in 1923 a fertilizer mixture composed of dried blood, superphosphate, and sulphate of potash and furnishing 32 pounds of nitrogen, 64 pounds of phosphoric acid, and 36 pounds of potash per acre was applied uniformly to all plots. Magnesium was applied in the form of sulphate at rates of 0, 12, 24, 36, and 48 pounds per acre. The results are summarized in Table 8.

The 1925 crop was destroyed by fire, so the results in Table 8 cover a period of only four years. The soil evidently contained a fair reserve of magnesium, for there have never been any clearly defined deficiency symptoms on the control plots. Nevertheless, it is apparent that up to 36 pounds of magnesium per acre has given appreciable increases in yield and value, while best results have been obtained with 24 pounds per acre. Even the 48-pound rate has only

slightly decreased the yield, and there have been no definite symptoms of toxicity. The calcium contained in 400 pounds of superphosphate apparently has prevented any toxic effects from the application of as much as 36 pounds of water-soluble magnesium.

TABLE 8.—*Effect of water-soluble magnesium when used in conjunction with superphosphate on yield and value of the tobacco crop at Oxford, N. C., during four different years*

Magnesium per acre applied in fertilizer (pounds)	Yields of tobacco (pounds per acre)					Value of crop (dollars per acre)				
	1923	1924	1926	1927	Average	1923	1924	1926	1927	Average
0.....	1,308	817	924	811	965	414	204	284	267	262
12.....	1,238	870	1,017	880	1,001	382	223	309	287	300
24.....	1,348	855	1,008	997	1,052	434	218	330	322	326
36.....	1,210	785	984	1,007	997	376	200	316	335	307
48.....	1,120	738	805	1,023	922	355	182	266	342	286

The experiments with less readily soluble forms of magnesium have been practically limited to the use of dolomitic limestone. A series of comparative tests with dolomitic limestone, calcitic limestone, and no lime—the limestones being applied in the drill each year at the rate of 1,000 pounds per acre and used in conjunction with different sources of potash—has been in progress since 1921. The details of these experiments have been reported in part elsewhere (5, 6). The plots receive as fertilizer a mixture of dried blood, superphosphate, and potash salts supplying 32 pounds of nitrogen, 64 pounds of phosphoric acid, and 36 pounds of potash per acre. The potash salts used are sulphate, muriate, sulphate of potash-magnesia, and kainite. Where muriate and sulphate of potash are used without calcium and with calcite, magnesium-deficiency symptoms have been severe. The magnesium contained in the other potash salts has prevented the deficiency symptoms. All plots receiving dolomitic limestone have remained free from the symptoms of magnesium deficiency. More recently the calcite plots are falling behind the unlimed plots in yield, suggesting that the calcite has the effect of depressing the solubility of the magnesium in the soil, in accordance with the findings of MacIntire and his associates (2). The point of special interest in the present connection is that after an annual application of 1,000 pounds per acre of dolomitic limestone for a period of eight years there is as yet no evidence of magnesium toxicity. As a matter of fact, recent pot tests indicate that dolomitic limestone may be used successfully as a medium for growing tobacco plants by simply applying a suitable nutrient solution. With an excess of calcium over the magnesium in dolomitic and magnesian limestones there is, perhaps, no reason for expecting toxic effects from the free use of such materials.

The present discussion centers around magnesium and calcium as plant nutrients and is distinct from the question of liming as such. The reaction of the soil, however, is of practical importance from the standpoint of both disease and nutrition conditions of the plant. The tobacco plant will tolerate a moderately acid soil reaction, but it occasionally happens that the acidity is excessive. In a series of fertilizer tests which have been in progress at the Oxford Tobacco Branch Station since 1913 there is a treatment consisting of ammo-

nium sulphate, superphosphate, and sulphate of potash in quantities to furnish 25 pounds of nitrogen, 64 pounds of phosphoric acid, and 80 pounds of potash per acre. Continued use of this mixture apparently has depleted the supply of alkaline earths in the soil and increased the acidity to the point where, in addition to marked symp-

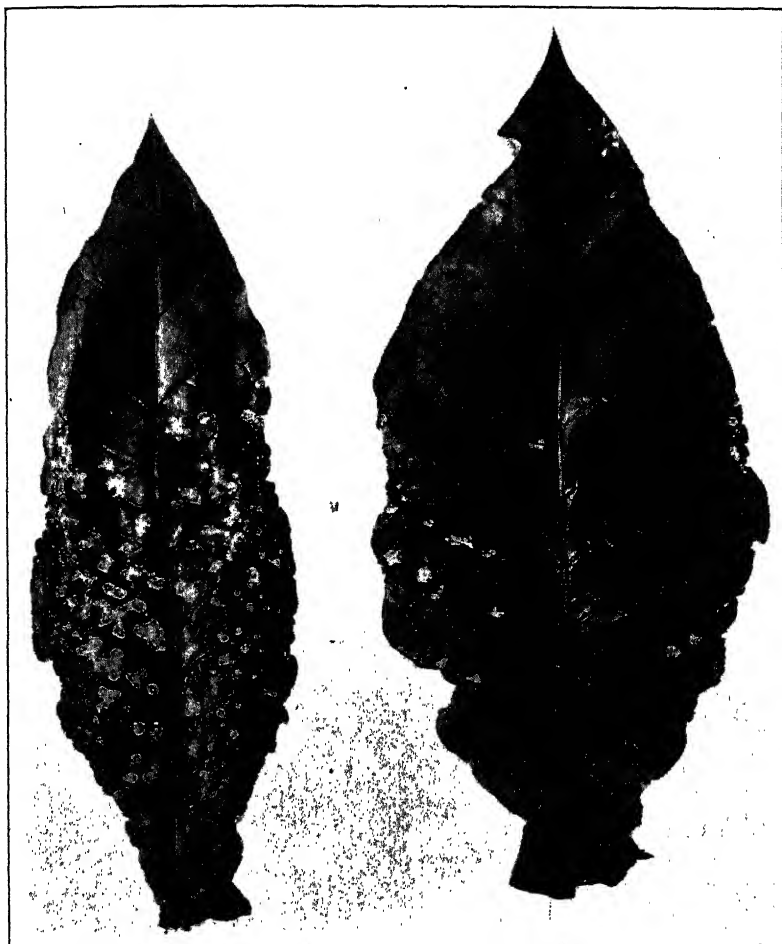


FIGURE 6.—Tobacco leaves from a plot fertilized for a period of years with ammonium sulphate, superphosphate, and sulphate of potash, without liming. The acidity of the soil has increased to the point where severe symptoms of manganese toxicity develop in the upper leaves of the plant, as here shown. The lower leaves of the plant also develop magnesium-deficiency symptoms. Calcitic limestone removes the manganese toxicity, while dolomitic limestone overcomes both troubles

toms of magnesium deficiency, there are in the upper leaves of the plant definite disease symptoms of a different character, probably due to manganese toxicity. These latter symptoms, which appear initially as reddish or brownish cankers on the upper leaves, are accentuated when a nutrient solution devoid of calcium is applied to pot cultures with soil from the plot in question. Leaves showing two stages of this effect are seen in Figure 6. In this case applications of dolo-

mitic limestone have given highly beneficial and profitable results, the average value of the crop for a period of five years having been increased from \$78 to \$174 per acre (5, 6). Where magnesium is not deficient, presumably any of the ordinary forms of lime may be used to correct excessive soil acidity.

On the other hand, it has been shown at the Connecticut Agricultural Experiment Station (4) that serious injury from the Thielavia root-rot disease of tobacco is likely to result if the soil acidity is reduced much below the point represented by the pH value 5.6, at least under the local conditions. Hence, the quantity of dolomitic limestone that may be safely or advantageously used on tobacco soils is not limited primarily by likelihood of toxic effects from its content of magnesium, but rather by the possibility of unduly lowering the acidity of the soil. Moreover, the reaction of the soil is a factor in determining the best form in which magnesium and calcium may be supplied.

CONCLUSION

Magnesium deficiency and calcium deficiency produce in the tobacco plant specific symptoms which are easily recognized under field conditions. Experimentation and observation have demonstrated that on a large proportion of the light-sandy and sandy-loam tobacco soils magnesium hunger is likely to develop in tobacco and even in other crops unless this element is supplied as a fertilizer. It now appears also that at least some of these soils require the addition of calcium as a fertilizer to prevent symptoms of calcium hunger and avoid serious crop injury. As long as farm manures and the old type of fertilizer, consisting largely of organic materials of plant and animal origin, were employed on these soils the necessary quantities of magnesium and calcium were usually supplied and no deficiency symptoms could be seen in the crop. With increased use of more highly concentrated inorganic and organic forms of nitrogen and potash in the fertilizer, magnesium deficiency has become an important factor in tobacco production on the soils in question. So long as superphosphate or other lime phosphate remains a leading constituent of the fertilizer there seems to be little likelihood of crop injury from calcium hunger. However, attempts to use very highly concentrated fertilizers consisting of mixtures of relatively pure chemicals supplying nitrogen, phosphoric acid, and potash but no magnesium or calcium have in some instances given decidedly unsatisfactory results. In such cases the difficulty has been overcome by adding small quantities of both magnesium and calcium. Addition of magnesium alone under these circumstances may result in evidences of magnesium toxicity. Potash apparently does not function effectively in preventing this toxicity.

SUMMARY

In this paper information in some detail is given concerning the symptoms and effects of magnesium deficiency in tobacco, the magnesium requirements of the crop, including comparison with certain other crop plants, and suitable means of supplying these requirements. Preliminary data also are given on symptoms of calcium deficiency in tobacco in close association with magnesium toxicity and the cal-

cium requirements of the crop, these data being derived largely from field experiments with highly concentrated fertilizers supplying nitrogen, phosphoric acid, and potash but no magnesium or calcium. Sharp distinction is made between supplying the magnesium and calcium requirements of the crop and the problem of liming the soil.

Under field conditions the outstanding feature of magnesium deficiency symptoms in tobacco (popularly known as sand drown) is a breaking down of both the green and the yellow chlorophyll pigments, which begins in the lower leaves of the plant and at the tips of the affected leaves. The bleached appearance of the leaves is distinctive. Magnesium hunger differs from potassium hunger in that the leaf surface ordinarily remains smooth, there is no downward curvature of the tips and margins, and specking or spotting resulting from localized breaking down of the tissue rarely occurs.

In magnesium deficiency the size of the leaves is somewhat reduced, while per unit area there is a decided decrease in dry matter and a well-defined increase in water content. There is a decrease in the carbohydrate content of the leaf. In the cured leaf the effects are most evident in the flue-cured type. Affected parts of the leaf are abnormally thin and nonelastic, have a papery texture, and usually show a dull, lusterless, light-brown color.

Analysis of the plant indicates that a minimum content of about 0.25 per cent magnesium or 0.4 per cent magnesia (MgO) in the leaf is required to prevent deficiency symptoms. With an available supply of magnesium no greater than the minimum requirements of the plant, the content of magnesium is somewhat higher in the upper than in the lower leaves, while the content in the stalk is about the same as the average for all leaves on the plant. With a more liberal supply of magnesium the highest percentage is found in the lower leaves and the content in the leaves as a whole greatly exceeds that in the stalk. On the basis of the above figures, the minimum requirements for a crop of 1,000 pounds leaf and 500 pounds stalks would be about 2.5 and 1.25 pounds, respectively, of magnesium (4 and 2 pounds MgO), or a total of about 6 pounds magnesia (MgO).

Experimentation and observation show that magnesium-deficiency symptoms are rather widely prevalent on some of the most important sandy and sandy-loam tobacco soils, especially in seasons of comparatively heavy rainfall. Chemical analysis shows that some of the soils are very low in magnesium, and, moreover, severe deficiency symptoms have been observed even on soils containing up to 0.24 per cent magnesium (0.4 MgO) soluble in strong acid.

Experience indicates that when applied in the drill 12 to 18 pounds of water-soluble magnesium (20 to 30 pounds MgO) per acre, and often less, will effectively prevent symptoms of magnesium deficiency. It has been shown also that 500 to 1,000 pounds per acre of dolomitic limestone applied either in the drill or broadcast will effectively control this trouble. Certain fertilizer materials, barnyard manure, and various crop residues contain beneficial quantities of magnesium.

Preliminary studies have shown that when highly concentrated fertilizers that contain no magnesium or calcium, such as a mixture of ammonium phosphate and nitrate of potash, are used on some of the sandy and sandy-loam soils the tobacco crop makes very poor growth. Addition of water-soluble magnesium to such a fertilizer

greatly stimulates growth, but a pathological condition develops in the upper portion of the plant and the leaves show striking abnormalities. By adding calcium along with the magnesium a normal crop is obtained.

It is difficult to distinguish clearly between the effects of magnesium toxicity and those due to calcium deficiency as such, but in any case an inadequate supply of calcium results in aborted leaf development in which there are large indentations in the margins and the tips are wanting. Preliminary studies indicate that the minimum content of calcium in the leaf required to prevent deficiency symptoms is in excess of 1 per cent, or some four or five times the magnesium requirement.

In the absence of an adequate supply of calcium, toxic effects may be expected from even comparatively small quantities of soluble magnesium, but the calcium contained in 400 pounds of superphosphate has effectively prevented toxic effects from 36 pounds of water-soluble magnesium applied as fertilizer. Dolomitic limestone, because of its calcium content, may be used freely without fear of inducing magnesium toxicity, the consideration limiting the quantity that may be advantageously used being a possible danger of unduly lowering soil acidity and thereby favoring the development of root diseases.

LITERATURE CITED

- (1) GARNER, W. W., McMURTREY, J. E., JR., BACON, C. W., and MOSS, E. G.
1923. SAND DROWN, A CHLOROSIS OF TOBACCO DUE TO MAGNESIUM DEFICIENCY, AND THE RELATION OF SULPHATES AND CHLORIDS OF POTASSIUM TO THE DISEASE. *Jour. Agr. Research* 23: 27-40, illus.
- (2) MACINTIRE, W. H., SHAW, W. M., and YOUNG, J. B.
1923. RECIPROCAL REPRESSION EXERTED BY CALCIC AND MAGNESIC ADDITIONS UPON THE SOLUBILITY OF NATIVE MATERIALS IN SURFACE SOIL. *Soil Sci.* 16: 449-464.
- (3) MEYER, D.
1910. DIE KALK-UND MAGNESIADÜNGUNG. 108 p. Berlin.
- (4) MORGAN, M. F., and ANDERSON, P. J.
1927. RELATION OF SOIL REACTION TO BLACK ROOTROT AND GOOD TOBACCO. *Conn. Agr. Expt. Sta., Tobacco Sta. Bul.* 8: 47T-49T, illus.
- (5) MOSS, E. G., McMURTREY, J. E., JR., and LUNN, W. M.
1927. FERTILIZER EXPERIMENTS WITH FLUE-CURED TOBACCO. *N. C. Dept. Agr. Bul.* June 1927, 40 p., illus.
- (6) ——— McMURTREY, J. E., JR., LUNN, W. M., and CARR, J. M.
1927. FERTILIZER TESTS WITH FLUE-CURED TOBACCO. *U. S. Dept. Agr. Tech. Bul.* 12, 59 p., illus.
- (7) MURWIN, H. F.
1929. THE EFFECTS OF MAGNESIA, SULPHUR AND CHLORINE ON THE GROWTH AND QUALITY OF TOBACCO. *Conn. Agr. Expt. Sta. Bul.* 299: 198-203.
- (8) WILLSTÄTTER, R., and STOLL, A.
1913. UNTERSUCHUNGEN ÜBER CHLOROPHYLL. *METHODEN UND ERGEBNISSE.* 424 p., illus. Berlin.

FIELD TESTS WITH TREATED SEED CORN¹

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INTRODUCTION

Much interest has developed in recent years concerning possible benefits to be derived from the treatment of seed corn with certain chemical compounds before planting. Although importance was originally attached to the question of growth stimulation, seed treatment has come to be regarded as chiefly a problem of disinfection of the seed to kill disease-producing organisms on the surface and to protect the planted seed from organisms in the soil which cause decay.

Following the intensive studies made during the last decade by a number of investigators, including Hoffer and Holbert (5, 7),² Durrell (3), Holbert, Burlison, Koehler, Woodworth, and Dungan (6), and others, concerning the seedling diseases of corn, it was but natural that investigation should turn to the possible control of these diseases by the use of fungicides. Such investigations have been fruitful, and it has been shown by Holbert, Reddy, and Koehler (8), Reddy, Holbert, and Erwin (13), Melhus, Reddy, Raleigh, and Burnett (12), and Clayton (2), that a number of seed-borne seedling-disease organisms are more or less subject to control by certain mercuric disinfectants. These organisms, chief among which are *Diplodia zeae*, *Gibberella saubinetii*, and *Basisporium gallarum*, also belong to the group causing dry rots of the ear. All of these have rather similar life histories and respond similarly to seed treatments. These organisms are all found to some extent in Nebraska, *Diplodia* being the most prevalent.

Much confusion exists among corn growers as to just what kind of corn diseases may be influenced by seed treatment and to what extent the quality of the crop may be benefited. Many have mistakenly gained the impression that seed treatment should prevent rotten, moldy, smutty, or otherwise diseased ears in the crop. In some years considerable losses are experienced from these ear rots. Especially is this true in seasons of slow growth and latematurity. Varieties of corn that are poorly adapted by virtue of being too late in ripening are also more subject to the development of ear rots late in the season than are well-adapted sorts.

The dry-rot disease organisms which may be expected to respond to seed treatment may each cause diseases of two kinds—seedling blights in the very early stages of plant growth, and stalk and ear rots in the more advanced stages. These rots have never been shown to be related to the seedling blights, and the two kinds of diseases may be regarded as independent, even though the same causal organism is involved.

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² Reference is made by number (italic) to "Literature Cited," p. 189.

Stalks, ear shanks, cobs, and grain may become infected during growth by these organisms, the spores of which are usually present in corn-crop residues of previous years. The amount of infection and severity of development of these stalk and ear rots vary both regionally and seasonally and to some extent with cultural practices and varietal type grown, but they are not influenced by the presence of organisms in the seed planted. Therefore no seed treatment need be expected to influence the percentage of diseased or rotted ears in the crop grown.

If seed from ears which have become infected as indicated above is planted, the other expression of disease, namely, seedling blight, may result from the viable seed so infected. Under conditions favorable for the development of the disease weakening or death of the seedling may result. It appears that serious injury is largely restricted to the seedling stage. Durrell (3, 4) has found that the diseases caused by *Diplodia* and *Basisporium* are not systemic, and the contrary has never been shown for *Gibberella*.

The primary purpose of the tests herein reported was to establish the general principles concerning the use of seed-corn treatments under farm conditions. The three subjects under investigation are as follows: (1) Effect of field-plot technic on the reliability of seed-treatment results; (2) effect of seed treatments on diseased seed as contrasted with farm-selected "planter-box" seed and disease-free seed; and (3) effect of seed treatment in relation to time of planting, as a protection against soil-borne organisms.

EXPERIMENTAL METHODS

The disinfectants used for the seed-corn treatments were all commercial compounds having some form of mercury as their active disinfectant principle. The materials employed were Uspulun, Bayer Dust, Semesan Jr. or Improved Semesan Jr., and Merko. All of these except Uspulun are dusts to be applied by standard methods at the rate of 2 ounces per bushel of seed. Uspulun is used as a one-fourth of 1 per cent solution in which the seed is soaked for approximately one hour.

The results reported in this paper were all secured at the agricultural experiment station at Lincoln, Nebr., with the exception of some from certain outlying cooperative experiments conducted in 1928. The tests extended over the 4-year period 1925 to 1928, inclusive. Investigations of the first two years, which have already been reported (11), were continued and supplemented, and all of the data are here summarized.

In those instances where it was anticipated that stand differences might result from treatment, multiple-row plots were employed to reduce the errors of plot competition, except in the case of certain studies in technic. The plots were commonly 13 rods long and were replicated two or more times. The plantings at the experiment station were done by hand, an equal number of seeds being dropped in each hill, thus permitting accurate field-stand determinations, which were made about three weeks after planting. The seed was spaced about 4 inches apart in the hill, which facilitated the counting of plants and suckers at maturity.

A rather complete set of agronomic notes was taken in connection with these experiments. These data are included in the accompanying tables to indicate the possible effect on growth of the disease or of the seed treatments.

COMPARATIVE EFFECT OF VARIATIONS IN FIELD-PLOT TECHNIC

In order that the results from seed-corn treatment experiments may be applicable, it is necessary that the methods of testing approach closely those that prevail on farms. When the stand of corn in a field is somewhat thinned by seedling diseases, the remaining plants may be expected to produce more heavily because of reduced competition. This increased yield may often largely or entirely compensate for the missing plants. Such adjustment takes place between plants within the same hill or between hills in the same row, or in adjacent rows (10). If the character of the field plots used for testing yields is such as to prevent a normal adjustment for missing plants, then correct comparable yields applicable to farm conditions can not be secured.

A special method study was conducted in 1928, comparing treated and untreated *Diplodia*-infected seed in single rows and in 3-row blocks. In the latter only the middle rows were harvested for yield determinations. A composite lot of diseased seed was divided into four lots. Three portions were treated, with Bayer Dust, Improved Semesan Jr., and Merko, respectively; the fourth remained untreated. The three lots of treated seed were planted consecutively in alternation with untreated seed, the entire series being replicated five times in both kinds of plots. With seed planted in this manner, the test was equivalent to 15 replications of treated and untreated seed in both alternating rows and alternating blocks. The results are given in Table 1.

TABLE 1.—Comparative results from planting treated and untreated *Diplodia*-infected seed^a of dent corn in alternating-row and alternating 3-row plots, 1928

ALTERNATING 1-ROW PLOTS

Treatment	Plots average	Field stand in—		Mature height	Percentage of plants—				Inferior mature plants	Diseased ears ^b	Yield of shelled corn per acre
		Spring	Fall		Barren	Lodged	Broken	With suck-ers			
	No.	P. ct.	P. ct.	In.					P. ct.	P. ct.	Bu.
Bayer Dust.....	5	80	79	110	21	2	33	2	4	2	25.5
Improved Semesan Jr.....	5	76	75	111	19	1	42	3	1	0	26.3
Merko.....	5	77	76	111	22	4	43	2	4	1	26.3
Total or average treated.....	15	78	77	110	21	2	39	2	3	1	26.0
Total or average untreated...	15	57	55	109	19	3	40	4	4	2	21.5

ALTERNATING 3-ROW PLOTS^c

	No.	P. ct.	P. ct.	In.	Barren	Lodged	Broken	With suckers	Inferior mature plants	Diseased ears ^b	Yield of shelled corn per acre
Bayer Dust.....	5	76	75	108	29	5	51	5	2	2	24.3
Improved Semesan Jr.....	5	79	79	109	31	2	37	2	2	1	24.6
Merko.....	5	75	73	109	26	3	44	1	3	2	24.6
Total or average treated....	15	75	76	109	29	3	44	3	2	2	24.5
Total or average untreated...	15	59	58	108	13	5	43	4	4	1	25.1

^a Funk 329 variety obtained from J. R. Holbert of the U. S. Department of Agriculture.

^b Throughout these tables and discussion "diseased ears" refers to ears visibly affected with dry rot.

^c Yields based on center row.

In this test it is assumed that correct relative results, free from the systematic error of adjoining plot competition between two unlike

stands, are obtained from the middle rows of 3-row plots. A much better stand resulted from the treated seed, 21 more plants being secured per 100 seeds planted in the single rows and 18 more in the 3-row plots. Expressed in another way: Treatment resulted in 37 and 31 per cent more plants, respectively, in the 1-row plots and the 3-row plots. This did not, however, prove to be of any particular advantage in the multiple-row plots, since the individual plants of the thinner stand were enabled to develop more favorably and thereby still fully occupy the land under the prevailing conditions. The untreated 3-row plots with fewer plants yielded 0.6 bushel, or 2 per cent, more per acre than did the treated plots. On the other hand, a correspondingly thin stand was unable to undergo a corresponding unhampered plant adjustment in the single-row plots because of the close competition with a heavier stand in immediately adjacent rows. Under these highly competitive conditions the heavier stand of the treated plots had an undue advantage, while the thinner stand of the untreated seed was at a marked disadvantage. This resulted in a much exaggerated yield effect in favor of seed treatment. Instead of the 0.6 bushel, or 2 per cent, decrease obtained in the 3-row plots, a 4.5 bushel, or 21 per cent, increased yield was obtained. This difference in favor of treatment represents a systematic experimental error of the test. This competition is also reflected in the effect on the percentage of barren plants under the two methods of testing. Incidentally it may be noted that the further loss in stand during the growing season was practically no greater for the untreated than for the treated seed.

These competition results correspond closely to those obtained by the writer (10) in 1914, when two planting rates, two plants and four plants per hill, were likewise compared in alternating rows and alternating 3-row blocks. In the latter case, unhampered by adjoining plot competition with a heavier stand, the 2-plant rate yielded 16 per cent more than the 4-plant rate, whereas in competing single rows the 2-plant rate yielded 18 per cent less. This same principle was exhibited during three years of testing, although the most productive stand naturally varies with the season and variety. The same principle will apply, though in lesser degree, to plots containing two or more rows unless border rows are discarded. Wherever corn is grown at a thick enough planting rate to give maximum yields, this principle of compensation between two adjoining rows differing in stand is quite likely to operate, and for this reason a statement of technic facilitates the interpretation and appraisal of experimental results.

COMPARATIVE EFFECTS OF SEED TREATMENT ON DIPLODIA-INFECTED SEED, FARM-SELECTED SEED, AND NEARLY DISEASE-FREE SEED CORN

TESTS IN 1927

Two lots of Diplodia-infected seed and one lot of nearly disease-free seed were obtained from Illinois for a seed-treatment test in 1927. A portion of each of these lots was treated with Bayer Dust and tested in comparison with untreated seed in duplicate 3-row plots 35 hills long. The corn was planted at the rate of three seeds per hill, which is the standard planting rate in the eastern part of Nebraska. The results are given in Table 2, together with a summary of the results from 17 lots of Nebraska farm-selected seed grown in an adjoining portion of the same field.

TABLE 2.—*Effect of mercuric seed treatment upon the crops from Diplodia-infected, nearly disease-free, and "planter-box" seed corn, 1927*
 [Plots consisted of 3 rows 35 hills long, in duplicate]

Source of seed ^a	Description of seed	Treatment	Field stand Per cent	Date in tassel	Mature height Inches	Percentage of plants—			Diseased ears Per cent	Shrinkage of ear corn Per cent	Shelling percent-age	Yield of shelled corn per acre Bushels
						Barren	Lodged	With suckers				
J. R. Holbert.....	Diplodia-infected.....	{Bayer Dust.....	89	July 20	96	3	13	12	1.0	6	83	62.2
B. F. Koehler.....	do.....	{None.....	80	do	96	4	11	14	.7	4	84	57.9
J. R. Holbert.....	Nearly disease-free.....	{Bayer Dust.....	85	do	92	5	25	15	1.2	7	84	64.7
17 Nebraska farmers.....	"Planter-box".....	{None.....	79	do	94	3	21	12	1.1	6	84	62.4
		{Bayer Dust.....	95	do	93	2	15	36	.8	5	83	66.7
		{None.....	95	do	91	4	10	40	.9	6	83	64.7
		{Bayer Dust ^b	85	July 28	87	4	21	20	1.2	6	83	53.9
		{None.....	87	do	87	5	21	21	1.4	6	83	55.0

^a The laboratory germination tests of this corn in the order listed were 94, 90, 90, and 93 per cent, respectively, for untreated seed.

^b 8 lots treated with Bayer Dust and 9 with Semesan Jr.; these data for "planter-box" corn taken from Table 4.

One sample of *Diplodia*-infected seed was improved 9 per cent in stand and 4.3 bushels per acre in yield by treatment, while nearly disease-free seed from the same source was unaffected as to stand but yielded 2 bushels more after treatment. The other sample of *Diplodia*-infected seed gave a 6 per cent better stand when treated and 2.3 bushels more grain per acre. An average loss in yield of 1.1 bushels per acre resulted from the treatment of farm-selected seed obtained from 17 eastern Nebraska farmers and grown in the same field. The field stand from this seed was improved 2 per cent. While these data are not very conclusive, they indicate that some betterment of stand and yield resulted from the treatment of severely *Diplodia*-infected seed under the rather favorable conditions of these tests.

TESTS IN 1928

A direct comparison was made in 1928 of the effects of mercuric disinfectants upon *Diplodia*-infected seed, nearly disease-free seed, and ordinary seed selected by farmers for home use. The tests were systematically replicated four times in 3-row plots of which only the middle rows were harvested for yields. Three seeds were planted in each hill. The results are summarized in Table 3.

Seed treatment increased the field stand 18 per cent for the diseased seed, lowered it 2 per cent for the nearly disease-free seed, and increased it 2 per cent for the farm-selected seed. The loss of plants between spring and fall was almost negligible and differed but little in the lots from treated and untreated seed. Averaging all treatments, the yield of the *Diplodia*-infected seed was increased 0.7 bushel per acre, as compared with 0.4 bushel decrease for the farm-selected seed and 0.8 bushel decrease for the nearly disease-free seed. There was no evidence of growth stimulation.

Seed treatment had no appreciable effect upon the number of inferior plants or diseased ears produced, even in the case of severe *Diplodia*-infected seed.

TABLE 3.—Comparative effects of various mercuric seed treatments upon the crops from *Diplodia*-infected seed, ordinary "planter-box" seed and nearly disease-free seed corn, 1928

FUNK 329, DIPLODIA-INFESTED SEED FROM ILLINOIS

Treatment	Plots averaged	Field stand in—		Date in tassel	Mature height	Percentage of plants—			Inferior plants in—		Diseased ears	Yield of shelled corn per acre *
		Spring	Fall			Barren	Lodged	Broken	With suckers	Spring	Fall	
Bayer Dust.....	Number	Per cent	Per cent	Aug. 9	Inches	28	6	51	7	Per cent	Per cent	Bushels
Improved Semesan Jr.	4	81	80	Aug. 9	108	30	2	33	2	6	3	28.9
Merko.....	4	77	79	do	109	24	4	51	1	7	3	27.2
		77	76	do	109					4	3	28.2
Total or average treated.....	12	78	78	do	109	27	4	45	3	6	3	28.1
Total or average untreated.....	4	60	58	Aug. 10	108	14	5	46	5	7	4	27.4

HOGUE YELLOW DENT, "PLANTER-BOX" SEED FROM NEBRASKA

Bayer Dust.....	4	92	87	Aug. 5	109	21	8	68	12	5	5	31.5
Improved Semesan Jr.	4	93	92	do	107	22	5	63	13	5	3	30.2
Merko.....	4	91	90	do	107	18	7	62	10	4	4	32.5
Total or average treated.....	12	92	90	do	108	20	7	64	12	5	4	31.4
Total or average untreated.....	4	92	92	do	107	23	6	61	7	5	3	31.8

FUNK 329, NEARLY DISEASE-FREE SEED FROM ILLINOIS

Improved Semesan Jr.	4	91	92	Aug. 8	110	24	4	58	5	5	3	24.8
Untreated.....	4	93	92	do	112	24	2	53	7	4	2	25.6

* Yields were based on the center rows of 3-row plots.

EFFECT OF SEED TREATMENT ON FARM-SELECTED SEED CORN

SEED SECURED FROM 14 COUNTIES IN EASTERN NEBRASKA

In the spring of 1927, 17 samples of farm-selected seed, prepared for home use, were obtained from farmers located in 14 different counties of eastern Nebraska. Each sample was divided into two portions, one being treated with either Bayer Dust or Improved Semesan Jr., as specified, while the other remained untreated. All lots were planted in a field of the experiment station farm which had been in corn the previous year. The plots consisted of three rows 35 hills long. Four consecutive plots were hand-planted to each sample of corn. The first two of these plots were planted, respectively, to treated and untreated seed at the standard rate of three seeds per hill, yields being based on the entire area planted irrespective of the stand secured. The third and fourth plots were likewise planted to treated and untreated seed, but at the rate of six seeds per hill, followed by thinning uniformly to three plants per hill. The thinning of seedlings was done systematically by position in the hill in order to avoid changing the normal proportion of strong and weak seedlings. In this case only those hills were harvested for the yield test which had a full stand.

The corn from all of the farmers was grown in consecutive plots in the field in the same manner. The field stand was determined by counting all seedlings per plot three weeks after planting and calculating their ratio to the number of seeds planted. The average moisture-free weight was determined for those seedlings removed at the time of thinning the one set of plots planted at a double rate.

The results from the two methods of testing are summarized separately in Table 4. It would seem that the general principle relative to need for the treatment of farm-selected seed can be best established by averaging the data from all lots of seed. No great importance can be attached to the results secured with an individual farmer's seed because of a lack of sufficient duplication to permit analysis in such detail. This may be illustrated by the fact that the effect of treatment on the seed of individual farmers ranged from an increase of 3.4 bushels to a decrease of 4.1 bushels in yield per acre. It would seem unjustified to attribute such variation in results to differential varietal response.

As an average for the 17 samples of farm-selected corn, planted at the rate of three seeds per hill, the untreated seed yielded 55 bushels and the treated seed 53.9 bushels per acre. Where the seed had been planted at a double rate and thinned to a normal stand of three plants per hill, the untreated seed averaged 56.9 bushels and the treated 56.5 bushels per acre. At the age of 3 weeks, the field stand averaged three more plants per 100 seeds planted for the untreated than for the treated seed. No material effects from treatment were observed on seedling vigor, date in tassel, mature stalk height, barren stalks, lodged stalks, suckers, 2-ear stalks, unsound ears, shrinkage of ear corn, or shelling percentage.

TABLE 4.—Summary of the effect of mercuric seed treatments upon the growth and yield of farm-selected "planter-box" seed corn obtained from 17 farmers located in 14 counties of eastern Nebraska, 1927

PLANTED THREE SEEDS PER HILL

[Plots consisted of three rows 35 hills long, in duplicate]

Treatment	Seed sources averaged	Field stand	Seedlings, 3 weeks old		Date in tassel	Mature height	Percentage of plants—				Diseased ears	Shrinkage of ear corn	Shelling percentage	Yield of shelled corn per acre
			Average weight	Average height			Barren	Lodged	With suckers	With 2-eared stalks				
Improved Semesan Jr.	Number	Per cent	Grams	Inches	July 29	Inches	4	23	23	3	Per cent	Per cent		Bushel
Untreated	9	84			do	89	4	25	23	2	1.7	7	82	54.5
Bayer Dust	9	87			July 27	88	4	18	13	2	.8	6	83	53.5
Untreated	8	86			July 28	85	5	10	19	2	2.0	7	83	54.5
Total or average treated	17	85			do	87	4	21	20	2	1.2	6	83	53.9
Total or average untreated	17	87			do	87	5	21	21	2	1.4	6	83	55.0

PLANTED SIX SEEDS PER HILL AND THINNED TO THREE PLANTS PER HILL

Improved Semesan Jr.	9	82	0.86	13.3	July 29	87	4	24	16	2	1.3	0	83	53.5
Untreated	9	85	.82	13.2	July 28	87	3	24	20	2	1.2	6	83	53.1
Bayer Dust	8	84	.94	13.8	do	86	4	27	17	1	1.3	6	83	53.5
Untreated	8	86	.90	13.6	do	84	3	26	14	2	2.0	4	83	54.0
Total or average treated	17	83	.90	13.6	July 29	87	4	26	17	2	1.3	6	83	56.5
Total or average untreated	17	86	.86	13.4	July 28	86	3	25	17	2	1.6	5	83	56.9

SEED SECURED FROM 20 LANCASTER COUNTY FARMERS AND TESTED INDIVIDUALLY

Seed corn collected from 20 Lancaster County, Nebr., farmers was included in seed-treatment tests at the experiment station in 1926 and 1927. Uspulun-treated and untreated seed of each man's corn were grown side by side in unduplicated 1-row plots 64 hills in length at the rate of 3 plants per hill. Moisture conditions were much too dry for corn in 1926 but very favorable in 1927.

TABLE 5.—Average effect of seed treatment with Uspulun upon the growth and yield of dent seed corn secured from 20 Lancaster County, Nebr., farmers and tested individually, 1926 and 1927

[Single-row plots, 64 hills in length]

Plant character	Results in 1926 with—		Results in 1927 with—		Average	
	Un- treated seed	Treated seed	Un- treated seed	Treated seed	Un- treated seed	Treated seed
Plots.....number.....	20	20	20	20	20	20
Field stand.....per cent.....	75	77	76	79	76	78
Plant height June 7.....inches.....	8.6	8.3				
Mature height.....do.....	74	74	95	95	85	85
Barren plants.....per cent.....	71	77	5	5	38	41
Lodged plants.....do.....	17	15	13	15	15	15
Suckers.....do.....	6	4	6	5	6	5
Shrinkage of ear corn.....do.....	11	12	10	9	11	11
Shelling percentage.....per cent.....	80	80	84	84	82	82
Diseased ears.....per cent.....			2	2		
Yield per acre.....bushels.....	7.7	7.6	70.2	70.7	39	39.2

The average results (Table 5) for the seed secured from all of the farmers are again regarded as the most reliable indication of the effects of seed treatment. Such averages for the two years show yields per acre of 39.2 bushels for the treated and 39 bushels for the untreated seed. A 2 per cent better field stand from treated seed in this test is offset by a 3 per cent decrease in the preceding test.

No significant differences are shown in the averages for barren plants, suckers, lodged plants, plant height, shrinkage of ear corn, shelling percentage, or percentage of unsound ears in the crop.

SEED SECURED FROM 30 LANCASTER COUNTY FARMERS AND PLANTED AS A MIXTURE

During each of the three years 1925-1927 seed selected for home use was secured from 30 Lancaster County, Nebr., farmers and mixed together for a general comparative test of the three commercial organic mercury compounds then available. Six systematically distributed 3-row plots, 64 hills long, were planted to each treatment during each season. The annual and average results are given in Table 6. Although the growth and yield varied greatly in the different years, no material effect from seed treatment was observed in any season. The differences for the various compounds were small and well within the limits of experimental error.

TABLE 6.—*Effect of various seed treatments upon the growth and yield of "planter-box" seed corn secured from 30 different Lancaster County, Nebr., farmers and planted in composite, 1925-1927*

[Plots consisted of 3 rows 64 hills long]

RESULTS FOR 1925

Treatment	Plots averaged	Field stand secured	Seedling height June 7	Mature height	Percentage of plants—			Ear corn		Yield of shelled corn per acre
					Barren	Lodged	With suckers	Shrinkage	Shelling	
	Number	Per cent	Inches	Inches				Per cent	Per cent	Bushels
Uspulun.....	6	79	-----	98	15	25	6	6	83	39.2
Semesan Jr.....	6	79	-----	99	16	20	5	9	83	39.5
Bayer Dust.....	6	79	-----	98	15	21	7	8	82	38.7
Total or average treated.....	18	79	-----	98	15	22	6	8	83	39.1
Total or average untreated.....	6	79	-----	98	15	22	7	7	83	39.4

RESULTS FOR 1926

Uspulun.....	6	74	8.4	74	68	18	5	11	79	7.4
Semesan Jr.....	6	74	8.6	74	68	19	5	11	80	6.9
Bayer Dust.....	6	72	8.5	73	60	19	7	11	80	7.3
Total or average treated.....	18	73	8.5	74	65	19	6	11	80	7.2
Total or average untreated.....	6	73	8.5	73	63	20	5	8	80	7.2

RESULTS FOR 1927

Uspulun.....	6	78	-----	99	5	15	6	11	84	71.5
Semesan Jr.....	6	74	-----	100	4	13	6	13	84	70.6
Bayer Dust.....	6	75	-----	101	4	16	7	13	85	72.7
Total or average treated.....	18	76	-----	100	4	15	6	12	84	71.6
Total or average untreated.....	6	77	-----	99	3	13	5	14	85	70.7

AVERAGE FOR THREE YEARS

Uspulun.....	18	77	-----	90	29	19	6	9	82	39.4
Semesan Jr.....	18	76	-----	91	29	17	5	11	82	39.0
Bayer Dust.....	18	75	-----	91	26	19	7	11	82	39.6
Total or average treated.....	54	76	-----	91	28	18	6	10	82	39.3
Total or average untreated.....	18	76	-----	90	27	18	6	10	83	39.1

On the basis of a 3-year average, the field stands secured from seed treated with Uspulun, Bayer Dust, Semesan Jr., and from untreated seed were 77, 75, 76, and 76 per cent, respectively, while the corresponding yields of shelled corn were 39.4, 39.6, 39.0 and 39.1 bushels per acre. Averaging all treatments, the yield was reduced 0.3 bushel in 1925, was unaffected in 1926, and was increased 0.9 bushel in 1927. This amounts to 0.2 bushel increase for all treatments during the three years. The average stand for the three years was the same from treated and untreated seeds.

EFFECT OF SEED TREATMENT ON THE YIELD OF FARM-SELECTED CORN WHEN TESTED IN DIFFERENT LOCALITIES

Seed corn obtained from seven different eastern Nebraska farmers was compared for yield with and without treatment on the experiment station farm at Lincoln and also 60 miles distant, at Valley, Nebr. The point of special interest in this test is the comparatively more favorable environment at Valley in respect to rainfall and subirrigation. At the experiment station the corn was planted by hand at the rate of three seeds per hill in duplicate 3-row plots. At Valley the corn was planted in duplicate 2-row plots by means of a corn planter set at a constant calibration. In all cases the treated and untreated seeds of each farmer was planted in adjacent plots.

TABLE 7.—Comparative results from the planting of treated "planter-box" dent corn seed at Lincoln and at Valley, Nebr., 1928^a

RESULTS AT LINCOLN, NEBR.

Treatment	Field stand	Plants per 20 rods	Percentage of plants—			Diseased ears	Yield of shelled corn per acre
			Smutty	Barren	Lodged		
Bayer Dust.....	<i>Per cent</i> 95	<i>Number</i> 269	17	32	62	<i>Per cent</i> 3	<i>Bushels</i> 27.5
Untreated.....	96	272	15	33	59	2	27.5

RESULTS AT VALLEY, NEBR.

Bayer Dust.....		219				0.6	62.3
Untreated.....		223				.2	62.7

^a This test was conducted in cooperation with the Agricultural Extension Service. The seed was collected from farmers in four different eastern Nebraska counties. Results represent averages for seed from seven farmers.

The average results are reported for each locality in Table 7. Practically identical stands and yields were secured at Lincoln from both treated and untreated seed. At Valley the application of dust to the seed reduced the rate of drop, and on an average approximately 2 per cent fewer plants grew in the treated than in the untreated plots. Treatment lowered the yield 0.4 bushel per acre.

For use in testing the effects of three different dust treatments at Valley, Nebr., in 1928, the seed from these seven farmers was mixed in equal proportions. This seed mixture was divided into four lots, three of which were treated, respectively, with Bayer Dust, Improved Semesan Jr., and Merko; the fourth remained untreated. The untreated seed was planted in alternation with the three lots of treated seed in 2-row plots by means of a corn planter. The three treated lots averaged 5 per cent lower stand and 3.3 bushels lower yield than the untreated seed. (Table 8.) The reduced yield may perhaps have been due in part to the effect of plant competition associated with the manner of testing here followed. Since the treatment affected the mechanical operation of the planter, thereby lowering the stand 5 per cent, error due to plot competition might be expected, as was pointed out in connection with Table 1.

TABLE 8.—*Effect of various seed-corn treatments upon the yield of ordinary farm-selected "planter-box" dent corn seed^a planted at Valley, Nebr.,^b 1928*

[The plots consisted of 2 rows, 10 rods long, and were planted in duplicate with a corn planter set at a constant drop]

Treatment	Plants per 20 rods	Diseased ears	Shelling percent- age	Shrink- age of ear corn	Yield of shelled corn per acre
	<i>Number</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Bushels</i>
Bayer Dust.....	212	0	82.9	6.7	56.4
Sernesan jr.....	215	0	81.3	8.1	55.4
Merko.....	191	0	80.7	7.3	54.9
Average.....	206	0	81.6	7.4	55.6
Untreated.....	216	0	81.3	7.7	58.9

^a This corn was a composite sample of "planter-box" seed from 7 farmers.^b This test was conducted in cooperation with the Agricultural Extension Service on the farm of J. L. Gilmore.

PROTECTION OF SEED AGAINST SOIL-BORNE ORGANISMS

It has recently been suggested by a number of workers that the use of these mercuric dusts on seed corn will protect the seed against soil-borne organisms which may cause rotting in the early spring, and universal treatment for this purpose is recommended. Very few data bearing on this question have, however, been published.

In order to secure information concerning this problem a combined date-of-planting and seed-treatment test was made during the 3-year period 1926 to 1928, inclusive. Farm-selected seed of two standard eastern Nebraska varieties, White Prize and Hogue Yellow Dent, were each treated with Uspulun during 1926 and 1927, and with Bayer Dust in 1928. Treated and untreated seed of each variety was planted in adjacent 2-row plots at rather uniform 10-day intervals, beginning either on the 15th or 25th of April, and ending June 15. The entire series was in duplicate. Since the normal planting season for this region extends from about May 5 to May 20, it is evident that the dates here included materially preceded and followed the usual time of planting. The object of this test was to determine whether the results of treatment might be influenced by the date of planting with the usual accompanying change in climatic conditions.

TABLE 9.—Comparative field stand secured from treated and untreated "planter-box" seed corn of two standard varieties, planted at seven different dates in the spring, 1926-1928

[Plots consisted of 2 rows 64 hills long, in duplicate]

RESULTS FOR 1926

Variety	Treatment	Percentage field stand secured from seed planted on the average planting dates indicated							Average stand ^a	Laboratory germination test
		Apr. 15	Apr. 25	May 5	May 15	May 25	June 5	June 15		
White Prize.....	(Uspulun.....	87	86	87	83	76	88	72	Per cent	Per cent
	(Untreated.....	87	87	86	83	77	85	72	83	98
Hogue.....	(Uspulun.....	79	82	81	81	80	75	69	82	97
	(Untreated.....	80	80	83	82	80	74	68	78	93
Average.....	(Uspulun.....	83	84	84	82	78	82	71	81	96
	(Untreated.....	84	84	85	83	79	80	70	80	96

RESULTS FOR 1927

White Prize.....	(Uspulun.....	67	63	64	77	69	61	67	88
	(Untreated.....	64	58	67	75	66	59	65	91
Hogue.....	(Uspulun.....	69	69	65	77	67	65	69	93
	(Untreated.....	68	69	71	77	66	66	70	95
Average.....	(Uspulun.....	68	66	65	77	68	68	68	91
	(Untreated.....	66	64	69	76	66	63	68	93

RESULTS FOR 1928

White Prize.....	(Bayer Dust.....	84	83	75	83	82	84	83	83	99
	(Untreated.....	79	85	79	83	82	81	80	81	98
Hogue.....	(Bayer Dust.....	89	92	80	92	89	89	88	88	98
	(Untreated.....	87	90	83	90	87	87	84	87	100
Average.....	(Bayer Dust.....	87	90	78	88	86	87	86	86	99
	(Untreated.....	83	88	81	87	85	84	82	84	99

AVERAGE FOR 3 YEARS

White Prize.....	(Treated.....	80	75	77	78	80	72	77	95
	(Untreated.....	79	74	78	78	77	70	76	95
Hogue.....	(Treated.....	81	77	79	82	77	74	78	95
	(Untreated.....	79	78	81	81	76	73	78	96
Average.....	(Treated.....	81	76	78	80	79	73	78	95
	(Untreated.....	79	77	80	80	77	72	77	96

^a Averages for last 6 planting dates only.

TABLE 10.—Comparative yields secured from treated and untreated "planter-box" seed corn of two standard varieties, planted at seven different dates in the spring, 1926-1928

[Plots consisted of 2 rows 64 hills long, in duplicate.]

RESULTS FOR 1926

Variety	Treatment	Yields in bushels of shelled corn per acre secured from seed planted on the average planting dates indicated							Average yield ^a
		Apr. 15	Apr. 25	May 5	May 15	May 25	June 5	June 15	
White Prize	Uspulun	5.3	6.0	6.2	9.9	14.5	19.4	13.4	Bushels 11.6
	Untreated	6.0	5.1	6.8	10.5	14.4	21.5	13.5	12.0
Hogue	Uspulun	7.3	7.8	8.6	9.6	13.1	22.6	19.7	13.6
	Untreated	6.6	5.9	7.1	11.6	14.0	25.1	19.3	13.8
Average	Uspulun	6.3	6.9	7.4	9.8	13.8	21.0	16.5	12.6
	Untreated	6.3	5.5	7.0	11.1	14.2	23.3	16.4	12.9

RESULTS FOR 1927

White Prize	Uspulun	-----	45.1	48.4	43.5	42.6	40.2	36.4	42.7
	Untreated	-----	45.8	46.4	45.7	42.8	38.0	36.1	42.5
Hogue	Uspulun	-----	50.2	49.2	48.9	51.8	49.8	44.8	49.1
	Untreated	-----	50.5	48.6	49.3	51.2	45.9	43.4	48.2
Average	Uspulun	-----	47.7	48.8	46.2	47.2	45.0	40.6	45.9
	Untreated	-----	48.2	47.5	47.5	47.0	42.0	39.8	45.3

RESULTS FOR 1928

White Prize	Bayer Dust	50.6	55.7	45.0	41.9	37.0	31.6	30.7	40.3
	Untreated	51.9	57.4	50.3	45.5	40.2	30.7	30.4	42.4
Hogue	Bayer Dust	53.1	59.4	64.7	55.0	45.9	41.6	37.2	50.6
	Untreated	56.0	65.0	64.4	50.6	41.1	44.6	36.3	50.3
Average	Bayer Dust	54.4	57.5	54.9	48.5	41.5	36.6	34.0	45.5
	Untreated	54.0	61.2	57.4	48.1	40.7	37.7	33.4	46.5

AVERAGE FOR 3 YEARS

White Prize	Treated	-----	35.6	33.2	31.8	31.4	30.4	26.8	31.5
	Untreated	-----	36.1	34.5	33.9	32.5	30.1	26.7	32.3
Hogue	Treated	-----	39.1	40.8	37.8	36.9	38.0	33.9	37.8
	Untreated	-----	40.5	40.0	37.2	35.4	38.5	33.0	37.5
Average	Treated	-----	37.4	37.0	34.8	34.2	34.2	30.4	34.7
	Untreated	-----	38.3	37.3	35.6	34.0	34.3	29.9	34.9

^a Averages for the last 6 planting dates only.

TABLE 11.—*Effect of seed treatments upon the performance of "planter-box" seed corn planted at six dates in the spring during three years*

[Plots consisted of two rows 64 hills long, in duplicate. Averages for Hogue and White Prize varieties, 1926-1928]

Treatment and date of planting	Field stand	Date in tassel	Plant height	Percentage of plants—				Un-sound ears	Shrink-age of ear corn	Shelling per-centage	Yield of shelled corn per acre
				Smuted	Barren	2-eared	Broken or lodged				
Untreated:	<i>Per cent</i>		<i>Inches</i>					<i>Per cent</i>	<i>Per cent</i>		<i>Bushels</i>
Apr. 25, 1926..	84	July 21	73	29	50	2	77	-----	8	70	5.5
Apr. 25, 1927..	63	July 27	90	9	3	3	11	2	8	83	48.2
Apr. 25, 1928..	88	July 22	107	5	3	3	46	1	5	85	61.2
Average..	78	July 23	90	14	19	3	45		7	79	38.3
Treated:											
Apr. 25, 1926..	84	July 21	70	31	32	4	72	-----	12	80	6.9
Apr. 25, 1927..	68	July 28	87	7	1	3	11	1	6	85	47.7
Apr. 25, 1928..	90	July 22	107	6	4	2	43	1	3	85	57.5
Average..	81	July 24	88	15	12	3	42		7	83	37.4
Untreated:											
May 5, 1926..	85	July 29	67	28	40	6	74	-----	10	78	7.0
May 5, 1927..	64	Aug. 2	90	8	2	4	10	2	6	83	47.5
May 5, 1928..	81	July 26	104	6	2	3	43	1	4	84	57.9
Average..	77	July 29	87	14	15	4	42		7	82	37.5
Treated:											
May 5, 1926..	84	July 29	68	32	45	4	74	-----	10	78	7.4
May 5, 1927..	66	Aug. 2	90	6	3	3	10	0	8	86	48.8
May 5, 1928..	78	July 26	104	5	2	3	46	1	4	84	54.9
Average..	76	July 29	87	14	17	3	43		7	83	37.0
Untreated:											
May 14, 1926..	83	Aug. 1	69	26	27	5	69	-----	10	75	11.1
May 14, 1927..	69	Aug. 4	91	8	2	2	11	2	7	84	47.5
May 14, 1928..	87	Aug. 1	111	3	3	2	43	0	3	86	48.1
Average..	80	Aug. 2	90	12	11	3	41		7	82	35.6
Treated:											
May 14, 1926..	82	Aug. 1	66	24	28	5	78	-----	10	78	9.8
May 14, 1927..	65	Aug. 4	90	6	3	2	11	3	7	83	46.1
May 14, 1928..	88	Aug. 1	111	6	3	1	42	0	7	85	48.5
Average..	78	Aug. 2	89	12	11	3	44		8	82	34.8
Untreated:											
May 25, 1926..	79	Aug. 6	73	25	20	4	72	-----	12	81	14.2
May 25, 1927..	76	Aug. 10	91	7	2	6	6	2	9	83	47.0
May 25, 1928..	85	Aug. 6	114	6	4	1	51	2	8	84	40.7
Average..	80	Aug. 7	93	13	9	4	43		10	83	34.0
Treated:											
May 25, 1926..	78	Aug. 6	70	23	17	5	66	-----	11	82	13.8
May 25, 1927..	77	Aug. 15	92	7	1	5	4	1	11	82	47.2
May 25, 1928..	86	Aug. 6	114	5	6	1	52	1	7	83	41.5
Average..	80	Aug. 9	92	12	8	4	41		10	82	34.2
Untreated:											
June 5, 1926..	80	Aug. 12	71	20	8	4	67	-----	13	83	23.3
June 5, 1927..	66	Aug. 14	92	4	2	4	6	3	14	81	42.0
June 5, 1928..	84	Aug. 12	101	4	5	2	32	2	11	83	37.7
Average..	77	Aug. 13	88	9	5	3	35		13	82	34.3
Treated:											
June 5, 1926..	82	Aug. 12	73	18	7	4	66	-----	11	81	21.0
June 5, 1927..	68	Aug. 14	89	3	1	5	4	3	12	82	45.0
June 5, 1928..	87	Aug. 12	101	5	5	2	37	2	13	84	36.6
Average..	79	Aug. 13	88	9	4	4	36		12	82	34.2

TABLE 11.—*Effect of seed treatments upon the performance of "planter-box" seed corn planted at six dates in the spring during three years—Continued*

Treatment and date of planting	Field stand	Date in tassel	Plant height	Percentage of plants—				Un-sound ears	Shrink-age of ear corn	Shelling percentage	Yield of shelled corn per acre
				Smuted	Barren	2-eared	Broken or lodged				
Untreated:	<i>Per cent</i>		<i>Inches</i>					<i>Per cent</i>	<i>Per cent</i>		<i>Bushels</i>
June 15, 1926.	70	Aug. 16	76	13	10	4	40	-----	17	79	16.4
June 15, 1927.	63	Aug. 25	101	4	1	2	6	2	19	80	39.8
June 15, 1928.	82	Aug. 18	101	6	9	2	32	2	15	82	33.4
Average.	72	Aug. 20	93	8	7	3	26	-----	17	80	29.9
Treated:											
June 15, 1926.	71	Aug. 16	76	13	6	4	40	-----	16	76	16.5
June 15, 1927.	63	Aug. 25	96	4	1	4	7	2	20	81	42.0
June 15, 1928.	86	Aug. 18	101	4	5	2	32	1	20	83	34.0
Average.	73	Aug. 20	91	7	4	3	26	-----	19	80	30.8
Untreated:											
1926 Average.	80	Aug. 4	72	24	26	4	67	-----	12	78	12.9
1927 Average.	65	Aug. 9	93	7	2	4	8	2	11	82	45.3
1928 Average.	86	Aug. 4	106	5	4	2	41	1	8	84	46.5
Average.	77	Aug. 6	90	12	11	3	39	-----	10	81	34.9
Treated:											
1926 Average.	80	Aug. 4	71	24	23	4	66	-----	12	79	12.6
1927 Average.	68	Aug. 10	91	6	2	4	8	2	11	83	46.1
1928 Average.	86	Aug. 4	106	5	4	2	42	1	9	84	45.5
Average.	78	Aug. 6	89	12	10	3	39	-----	11	82	34.7

TABLE 12.—*Maximum, minimum, and mean daily air temperatures in °F.° during 10-day periods following the corn-planting dates reported in Tables 9, 10, and 11; 1926, 1927, and 1928*

Item	Average daily air temperature on dates included in period						
	Apr. 15 to Apr. 24	Apr. 25 to May 4	May 5 to May 14	May 15 to May 24	May 25 to June 4 ^b	June 5 to June 14	June 15 to June 24
1926:							
Maximum.....	69.8	76.1	67.6	79.8	83.2	87.2	77.3
Minimum.....	42.7	46.8	51.6	54.0	60.0	62.8	56.4
Mean.....	56.2	61.4	59.9	66.6	71.5	75.0	67.0
1927:							
Maximum.....	56.4	73.6	67.7	78.0	71.2	75.5	79.2
Minimum.....	40.0	50.5	46.5	56.4	51.8	55.9	59.3
Mean.....	48.1	62.0	57.2	67.1	61.5	65.7	69.2
1928:							
Maximum.....	58.3	70.1	76.2	76.4	75.6	76.5	78.4
Minimum.....	34.3	42.4	51.5	54.3	53.5	55.6	55.6
Mean.....	46.3	56.1	63.9	65.3	64.6	66.0	69.4
Normal mean.....	53.3	58.2	59.5	62.8	66.3	70.9	72.8

^a These temperature data are of interest in connection with date-of-planting tests reported in Tables 9, 10, and 11.

^b 11-day period.

The field stands secured at each planting date are reported separately for each variety and are also summarized for the 3-year period in Table 9. The corresponding acre yields of shelled corn are reported

in Table 10. The results secured each year with respect to various agronomic characters of the crop are summarized for the different planting dates in Table 11. The maximum, minimum, and mean daily air temperatures that prevailed during the 10-day periods immediately following the dates of planting in 1926 to 1928 are shown in Table 12.

The maximum deviation in stand obtained on any one planting date ranged from an increase of 5 per cent to a decrease of 6 per cent following treatment. When both varieties were averaged for the three years, the maximum deviation in stand between treated and untreated seed on any one planting date was 2 per cent, with a grand average of 1 per cent in favor of treatment. Considering the 6 dates as an average for the three years, the treatment gave a better stand on 3 dates, a reduced stand on 2 dates, and had no effect on 1 date.

Considering the grain yields for the individual years at the various dates, the maximum deviation ranged from an increase of 4.8 bushels per acre in favor of treatment to a decrease of 5.6 bushels per acre, likewise following treatment. Averaging both varieties for the three years, the maximum deviation in yield between treated and untreated seed at any one planting date was 1.2 bushels, with a grand average of 0.2 bushel in favor of the untreated seed. By comparing the individual dates averaged for the three years, the treated corn was found to yield most for the fifth and seventh and least for the other planting dates.

It is apparent from Table 11 that almost identical results were obtained from treated and untreated seed with respect to such characters as date of tasseling, plant height, barrenness, lodging, unsound ears, shrinkage and shelling percentage of ear corn after husking, percentage of field stand, and yield of grain per acre. From these data it does not appear that these mercuric compounds serve in an important degree as a protection of the seed from soil-borne organisms.

Smut counts, included in Table 11, give further evidence that corn smut is not influenced by seed treatment. Similar results from smut counts were obtained in all of the experiments herein reported.

DISCUSSION

The question may arise whether the conditions that influence the development of seedling diseases at the experiment station are representative of those that generally prevail in this State. Results with viable seed corn known to be heavily infected with specific ear-rot organisms indicate injurious effects on stand corresponding with those reported for Illinois and Iowa, where both climate and soil are regarded as conducive to the development of these organisms. A comparative field-plot test with such seed systematically replicated 30 times, in 1928, showed an average increase of 18 per cent in field stand as a result of treatment with mercuric fungicides. (Table 1.) In another test in 1928 (Table 2) the stand from nearly disease-free seed had 52 per cent more plants than the stand from Diplodia-infected seed of the same variety. In 1927 duplicate tests gave average field stands of 95 and 80 per cent, respectively, for nearly disease-free and Diplodia-infected seed. Treatment of the diseased seed improved the stand 9 per cent.

In 1922 a comparative replicated field test made by the writer (10) showed typical inferior stands when diseased seed was used, as follows: *Diplodia*-infected seed and *Fusarium*-infected seed gave field stands only 64 and 96 per cent as large as those from nearly disease-free seed. These results again were typical of those that have commonly been described for the State of Illinois, and lend confidence to the belief that these Nebraska Experiment Station conditions are responsive to both the disease organisms and to the mercuric fungicides. It is concluded that results obtained from seed treatments at the experiment station are applicable elsewhere in the State.

It is evident that inferior stands may be expected from seed that is severely infected with dry-rot seedling-disease organisms. It is also apparent that the loss of stand resulting from the use of such seed may be materially lessened through treatment with some one of the mercuric seed-corn disinfectants. However, such infected seed, even though treated, is likely to be definitely inferior, from the standpoint of field stand, to untreated nearly disease-free seed and to representative farm-selected seed. Whether the differences in stand due to either the disease or the treatment have any material effect upon yield depends upon the season and the actual stand obtained. This phase of the problem has been constructively analyzed by Clayton (1) in connection with a review of experiments pertaining to *Diplodia* dry rot of corn. He concludes that no differences in yield resulted from the use of diseased seed when it was planted thick enough to compensate for the reduced germination.

In view of the inferior stands secured in 1927 and 1928 from seed infected with *Diplodia* and in 1922 from seed infected with *Diplodia* and *Fusarium* (10), it becomes apparent that the germinator test described by the writer (9, 11) and patterned after that of Holbert and Hoffer (?) was unreliable as an index of infection with dry-rot disease organisms. In these earlier germinator tests a large number of seed ears were classified as diseased and disease-free without an attempt being made to identify the disease organisms. When planted, both groups gave approximately equal stands and equal yields during a 6-year period.

The failure to secure improved stands or improved yields during the last four years from the treatment of representative farm-selected seed collected from a large number of Nebraska farmers is evidence that these seedling dry rots are exerting little influence as seed-borne diseases in this State. This is quite at variance with the conclusions reached in Iowa by Melhus, Reddy, Raleigh, and Burnett (12), who show increases of 2.2 to 4.6 bushels per acre from the use of these commercial dust treatments applied to a local sample of farm-selected seed in each of five counties of the State in 1927. In Illinois also increases ranging from 2.1 to 5.4 bushels are reported for well-selected but untested seed, by Holbert, Reddy, and Koehler (8).

The failure of field stands and yields secured from farm-selected seed to be materially benefited by mercuric seed treatments irrespective of the time or favorableness of planting conditions is again at variance with the conclusions reached in Iowa and Illinois by the investigators last cited.

If the various results for these three States are typical, it follows that farm-selected seed planted in Nebraska is less subject to infec-

tion with dry-rot seedling-disease organisms than is farm-selected seed in Iowa and Illinois. Climatic and soil conditions in Nebraska are perhaps also less conducive to development of the seed-borne and soil-borne organisms which attack the planted seed and the young seedling. It is not known to what extent differences in plat technic may have influenced the relative yields where stands were affected by seed selection or treatment. That results and conclusions may vary with the experimental procedure has here been demonstrated.

It may be concluded that Nebraska-grown seed ears selected on the basis of sound and mold-free appearance are generally not appreciably benefited in production by treatment with these mercuric fungicides.

SUMMARY

Four commercial mercuric seed-corn treatments were tested under field conditions at the Nebraska Agricultural Experiment Station during the 4-year period 1925 to 1928, inclusive. These consisted of one liquid fungicide, Uspulun, and three dust compounds, Bayer Dust, Semesan Jr., or Improved Semesan Jr., and Merko. Field conditions at the station proved favorable to the development of the *Diplodia* seedling disease of corn when severely infected seed was planted, but at least partial control followed the treatment of such seed, the various treatments giving similar results. On the other hand farm-selected seed obtained from Nebraska growers was not significantly benefited by treatment.

Conclusions reached by a number of investigators elsewhere regarding the nonsystemic nature of the *Diplodia* dry-rot disease were substantiated in 1928 by direct comparison of the seed value of *Diplodia*-infected seed and nearly disease-free seed selected from the same variety. Although the seed infection curtailed the field stand 35 per cent, it caused no significant deterioration in plants or serious losses between spring and maturity. It did not greatly affect the growth of the plants or the proportion of inferior plants or diseased ears produced. The acre yield from the diseased seed was 1.8 bushels greater, probably due to the advantage of a somewhat thinner stand.

In a thoroughly replicated test in 1928, the three dust compounds improved the field stand from *Diplodia*-infected seed on an average 18 per cent and lowered the yield per acre half a bushel when tested in multiple-row plots. Failure to increase yield or to materially influence the growth, health, or grain quality of the surviving plants is evidence that the benefit from these seed treatments was limited to the germination and seedling stage of development. In a corresponding test made in alternating single-row plots, the same general results were obtained except that the treated seed yielded 4.5 bushels per acre more than the untreated. This difference in effect on yield as related to character of test plot, represents an experimental error of 5 bushels due to plot competition between unlike stands in the case of the 1-row plots. This suggests the necessity for knowing the details of plot technic in order that data may be properly appraised.

In 1927 seed treatment increased the field stands of two lots of *Diplodia*-infected seed an average of 8 per cent and the grain yield an average of 3.3 bushels per acre. No other significant effects were noted.

A total of 141 samples of farm-selected seed were tested for response to seed treatment during the 4-year period. Planting was done each

year during the normal planting season except in a special test during three years in which the seven planting dates materially overlapped the normal season. When the effects of treatment as summarized for the various experiments with farm-selected seed are prorated in accordance with the number of samples of corn represented, it is found that the field stand was increased an average of 0.28 per cent and the yield was increased 0.03 bushel per acre. This slight response to the mercuric seed disinfectants shows how little damage results from dry-rot infection of farm-selected seed under normal conditions in Nebraska. There have been no significant practical effects from the seed treatment of 141 samples of farm-selected corn upon such characters as stand, vigor and size of growth, earliness of maturity, percentages of smutty, barren, lodged, broken, and diseased plants, percentage of diseased ears, shrinkage and shelling percentage of the ear corn, and yield of grain per acre.

No beneficial effect was found to result from disinfecting seed corn to protect it from soil-borne organisms under field conditions associated with both abnormally early and late planting.

LITERATURE CITED

- (1) CLAYTON, E. E.
1927. DIPLODIA EAR-ROT DISEASE OF CORN. *Jour. Agr. Research* 34: 357-371.
- (2) ———
1928. INCREASING STANDS FROM VEGETABLE SEEDS BY SEED TREATMENT. *N. Y. State Agr. Expt. Sta. Bul.* 554, 16 p., illus.
- (3) DURRELL, L. W.
1923. DRY ROT OF CORN. *Iowa Agr. Expt. Sta. Research Bul.* 77, p. [346]-376, illus.
- (4) ———
1925. BASISPORIUM DRY ROT OF CORN. *Iowa Agr. Expt. Sta. Research Bul.* 84, p. [138]-160, illus.
- (5) HOFFER, G. N., and HOLBERT, J. R.
1918. SELECTION OF DISEASE-FREE SEED CORN. *Ind. Agr. Expt. Sta. Bul.* 224, 16 p., illus.
- (6) HOLBERT, J. R., BURLISON, W. L., KOEHLER, B., WOODWORTH, C. M., and DUNGAN, G. H.
1924. CORN ROOT, STALK, AND EAR ROT DISEASES, AND THEIR CONTROL THRU SEED SELECTION AND BREEDING. *Ill. Agr. Expt. Sta. Bul.* 255, p. 239-478, illus.
- (7) ——— and HOFFER, G. N.
1920. CONTROL OF THE ROOT, STALK, AND EAR ROT DISEASES OF CORN. *U. S. Dept. Agr. Farmers' Bul.* 1176, 24 p., illus.
- (8) ——— REDDY, C. S., and KOEHLER, B.
1928. CHEMICAL-DUST SEED TREATMENTS FOR DENT CORN. *U. S. Dept. Agr. Circ.* 34, 6 p.
- (9) KIESSELBACH, T. A.
1922. CORN INVESTIGATIONS. *Nebr. Agr. Expt. Sta. Research Bul.* 20, 151 p., illus.
- (10) ———
1923. COMPETITION AS A SOURCE OF ERROR IN COMPARATIVE CORN YIELDS. *Jour. Amer. Soc. Agron.* 15: 199-215.
- (11) ———
1927. FIELD EXPERIMENTS WITH SEED CORN TREATMENTS AND CROP STIMULANTS. *Nebr. Agr. Expt. Sta. Bul.* 218, 15 p., illus.
- (12) MELHUS, I. E., REDDY, C. S., RALEIGH, W. P., and BURNETT, L. C.
1928. SEED TREATMENT FOR CORN DISEASES. *Iowa Agr. Expt. Sta. Circ.* 108, 16 p., illus.
- (13) REDDY, C. S., HOLBERT, J. R., and ERWIN, A. T.
1926. SEED TREATMENTS FOR SWEET-CORN DISEASES. *Jour. Agr. Research* 33: 769-779, illus.

UTILIZATION OF CALCIUM BY THE GROWING CHICK¹

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INTRODUCTION

Considered from the quantitative standpoint, calcium is the most important mineral element involved in chick nutrition. With relatively little effort the most common forms of calcium can be changed to other forms should these be found to serve the purposes of nutrition more efficiently. The fundamental problem with reference to calcium is therefore one of obtaining proper assimilation and fixation. Calcium salts, whether soluble or insoluble, appear to be fixed in the bone tissue with considerable difficulty and our problem is one not simply of providing calcium but rather of providing it in a form and in combination with other factors which permit absorption and deposition. This paper presents the results of a study of the assimilation and utilization of calcium by growing chicks when this element was furnished in several different forms.

EXPERIMENTAL PROCEDURE

As a basal ration for the chicks used in the experiments reported in this paper a mixture was chosen that included corn, wheat, and milk proteins. This ration has many points in common with the mixtures used by thousands of practical poultry producers. Yeast and cod-liver oil were added to insure a sufficient supply of vitamins B and D, and sunshine was available to all lots in a screen-inclosed concrete-floored runway in front of each brooding unit. Steam and electric brooders were used for maintaining the proper temperatures for growth. The basal ration employed in the first series of experiments consisted of the following: Yellow corn meal, 50 per cent; shorts, 24 per cent; casein, 10 per cent; blood meal, 5 per cent; yeast, 3 or 4 per cent as indicated for each series; cod-liver oil, 2 per cent; and starch, 5 or 6 per cent as indicated for each series.

The starch was included in the above ration so that additions of minerals could be made by replacing an equivalent amount of starch without changing the vitamin or protein plane. This basal ration is very low in ash elements, containing only 0.174 per cent of calcium and 0.495 per cent of phosphorus. It was planned to be complete with respect to proteins, vitamins A, B, and D, and was very palatable, so that all requirements for energy were easily satisfied. The protein plane of the basal ration was 23.75 per cent. It was offered as an all-mash ration, available to the birds at all times. Ordinary tap water was used, and this contributed a small amount of calcium in addition to that contained in the ration. The water contained from 50 to 60 parts of CaO per million, and from observations made

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it is estimated that about 3,600 gm. of water was consumed per chick during the 8-week period of each experiment. About 0.18 gm. of calcium per chick may therefore have been added to the available supply from this source.

Single-comb White Leghorn chicks hatched from the college flocks were used for these experiments. Each chick was banded and weighed individually at the beginning of the experiment and biweekly thereafter. All other environmental conditions except length of day were standardized, but since growth rate is very markedly influenced by the length of day, no attempt is made to compare the growth rates of any chicks other than those which were fed at the same time in parallel pens in the chick nutrition laboratory.

Sex was determined on all lots sufficiently developed sexually to make accurate determinations possible. The growth curves in the graphs represent weighted averages of both sexes. This seemed to be

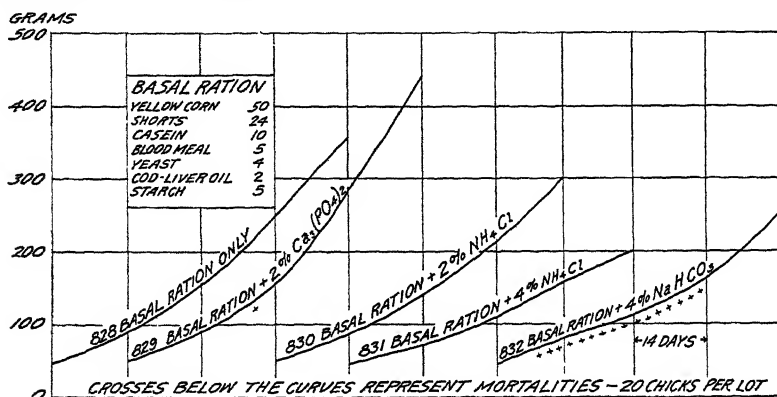


FIGURE 1.—Growth of chicks increased by a calcium supplement and inhibited by the addition of certain other minerals to the basal ration

the most accurate method of presenting the results. From 20 to 40 chicks were used in each lot, the same number of chicks being used in each pen in each series of experiments.

MINIMUM CALCIUM REQUIREMENTS

Until quite recently the prevailing opinion with reference to the mineral requirements of the growing chick has been that safety lies in providing an excess of essential mineral constituents. The soundness of this conclusion has however, been disproved by the experiments of Mussehl, Blish, and Ackerson (5).² Anticipating that evidence would be obtained of disturbed metabolism because of mineral deficiencies in the basal ration, even when vitamin D and radiant energy requirements were satisfied, the writers placed 20 vigorous Single-comb White Leghorn chicks in each of a number of pens and restricted one lot entirely to the basal ration already referred to, which is very low in mineral constituents.³ All 20 chicks survived and made reasonably good growth, though not so good as the chicks

² Reference is made by number (italic) to "Literature Cited," p. 198.

³ The ration received by lot 828 contained 2.64 per cent total ash material, of which 0.244 per cent was CaO, 1.132 per cent was P₂O₅, and 0.1663 per cent was Mg.

in a parallel pen receiving 2 per cent of tricalcium phosphate in addition. The growth curves for lots 828 and 829 in Figure 1 and of lots 845 and 846 in Figure 2 indicate the beneficial effect of adding 2 per cent of $\text{Ca}_3(\text{PO}_4)_2$ to this particular base. Curves 828 and 845, however, demonstrate the ability of the chick to exist on a very low plane of calcium intake for at least eight weeks provided conditions for assimilation and fixation are favorable.

OPTIMUM ADDITIONS OF TRICALCIUM PHOSPHATE

When it was observed that 2 per cent of tricalcium phosphate added to the basal ration had a beneficial effect on the growth rate of chicks, it was decided to add increasing amounts to determine if possible the optimum point with respect to these additions. The growth curves for lots 846, 847, and 848, as given in Figure 2, indicate

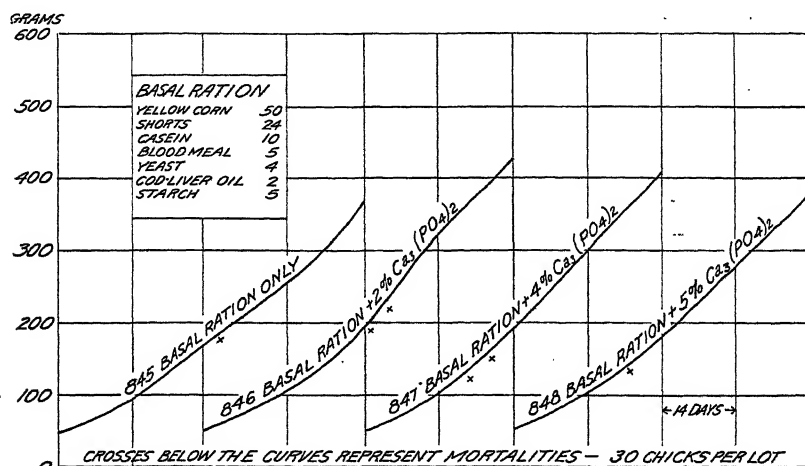


FIGURE 2.—The effect of increasing amounts of calcium upon the growth of chicks, when supplied in the form of the phosphate

no advantage from the addition of more than 2 per cent. The average growth rate of chicks receiving 4 and 5 per cent, respectively, of $\text{Ca}_2(\text{PO}_4)_3$ was not so good as for lot 846 receiving only 2 per cent. This is in agreement with experimental work previously reported (5) which indicated an excess of mineral elements to be undesirable.

THE EFFECT OF ACID-BASE CHANGES ON MINERAL METABOLISM

Changes in the acid-base values of the ration are believed to influence the assimilation and fixation of calcium and phosphorus.

Shohl, Bennett, and Weed (7) observe from the analyses of the bones of experimental animals that the ash deposition is greatest with neutral diets, smaller with alkaline diets, and least with acid diets. Metabolism studies also indicated the largest retentions of calcium and phosphorus on neutral diets. Zucker, Johnson, and Barnett (10) report the production of rickets in rats on a diet containing an excess of base over acid. When they substituted CaCl_2 for Ca lactate in equivalent amounts they increased the acidity of the diet. The bones of the experimental animals on the latter ration were nearly

normal or showed only mild rickets. The addition of 2 per cent NH_4Cl , they stated, prevented the development of rickets. They conclude that a diet, which with respect to balance between calcium and phosphorus should not lead to rickets, may do so when there is brought about a lessening of the acidity of the intestinal tract. Jones (3), using dogs as experimental animals, concludes that normal animals can well tolerate a certain excess of alkali in diets both high and low in phosphorus, but if the amount is sufficiently increased resorption of bone material occurs. Neutralization of the excess of alkali again initiates healing of the rachitic bone tissue.

Just how much tolerance the chick may have for variations of the acid-base content seems to be a matter of considerable importance, and experiments were planned to obtain information on this point. Figure 3 shows the effect of 2 per cent additions of NH_4Cl , NaHCO_3 ,

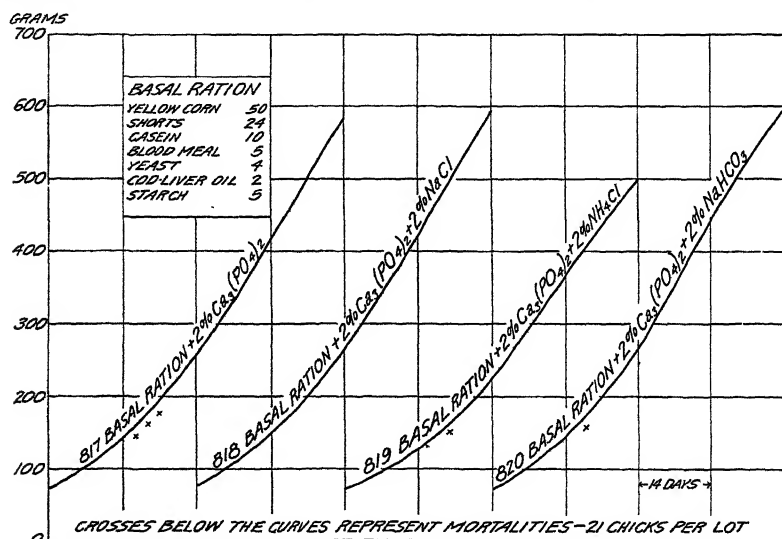


FIGURE 3.—The effect of a tricalcium phosphate supplement upon the growth of chicks, when supplied alone and in combination with various other minerals

and NaCl . With the basal ration used, 2 per cent additions of NaHCO_3 and 2 per cent of NaCl were evidently tolerated with no appreciable effect on the growth rate or well-being of the chicks. The addition of 2 per cent of NH_4Cl , however, had a very definite inhibitory effect on the growth rate, though the mortality rate was very low even on this ration, as shown by curve No. 819.

The curves for lots 831 and 832 in Figure 1 show that 4 per cent additions of the acid and base factors referred to proved very disastrous. The mortality rate of the chicks on the alkaline ration was especially high, but the growth rate of the survivors was somewhat higher than that of the chicks on the strongly acid ration.

Figure 4 shows an almost perfect repetition of the results reported in Figure 3, curves 817, 819, and 820, with a large number of chicks on each ration. Apparently with this particular basal ration the alkalinity contributed by 2 per cent of NaHCO_3 was tolerated better than was the acidity resulting from the addition of 2 per cent of NH_4Cl .

THE RELATIVE AVAILABILITY OF CALCIUM SALTS

Growth curves for lots 828 and 829 (fig. 1) and for lots 845 and 846 (fig. 2) show that the basal ration used could be improved by the addition of calcium in the form of tricalcium phosphate. An interesting question presents itself: Is there a difference in the availability of calcium in the various forms in which it is most easily furnished? Using rats as the experimental animals, Steenbock, Hart, Sell, and Jones (8) added calcium lactate, calcium carbonate, calcium phosphate, calcium sulphate, and calcium silicate at approximately the same plane to the same basal ration. They concluded that no difference in availability of these particular salts exists when they are fed in liberal amounts.

Since rats and birds are known to have somewhat different nutritional requirements, the writers have undertaken to obtain information on the utilization of various calcium

salts by the growing chick. Tricalcium phosphate, calcium lactate, calcium sulphate, calcium chloride, and calcium carbonate were compared at exactly the same calcium plane, this being the amount of each of the salts needed to contribute 1 per cent of calcium to the ration. To the ration of one lot (872) was also added 3 per cent of salt mixture No. 185 (McCollum)⁴ to determine whether the growth

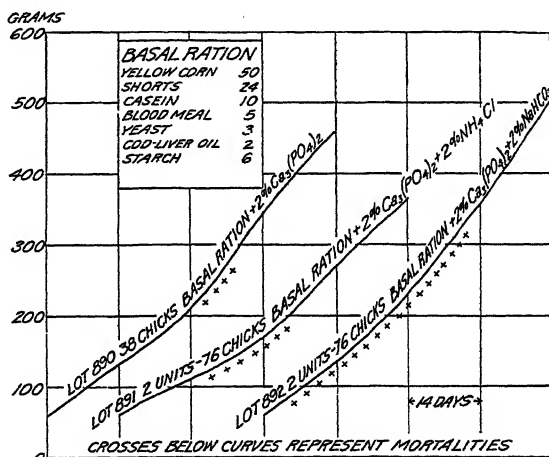


FIGURE 4.—The effect of a tricalcium phosphate supplement upon the growth of chicks when supplied alone and in combination with other minerals

rate could be improved by adding a complex inorganic mixture. The growth curves shown in Figures 5, 6, and 7 indicate that, when added at the same plane, the tricalcium phosphate was better utilized than were the other salts. Calcium carbonate and calcium lactate were quite well utilized, but calcium sulphate and calcium chloride had a decidedly inhibitory effect on the growth rate. Whether this was due to a change in the reaction of the ration on addition of the latter salts must remain a matter of speculation. One can from this evidence conclude that there is a difference in the utilization of the different forms of calcium, and that tricalcium phosphate, calcium carbonate, and calcium lactate are most readily assimilated and fixed.

DOES MAGNESIUM INHIBIT CALCIFICATION?

One finds in the literature on calcium metabolism the suggestion that magnesium salts interfere with calcium fixation. Many of the limestones used for poultry feeding contain appreciable amounts of

⁴ Salt mixture No. 185 contains the following ingredients expressed in terms of percentage: NaCl, 4.6; MgSO₄, 7.1; NaH₂PO₄·H₂O, 9.3; K₂HPO₄, 25.7; CaH₂(PO₄)₂·H₂O, 14.6; ferric citrate, 3.1; calcium lactate, 35.1.

magnesium. It is therefore important to know whether the amount of magnesium which may be included when impure limestone is used is really detrimental.

Shelling, Kramer, and Orent (6) find that calcification occurs most readily in the absence of magnesium. Haag and Palmer (1) state

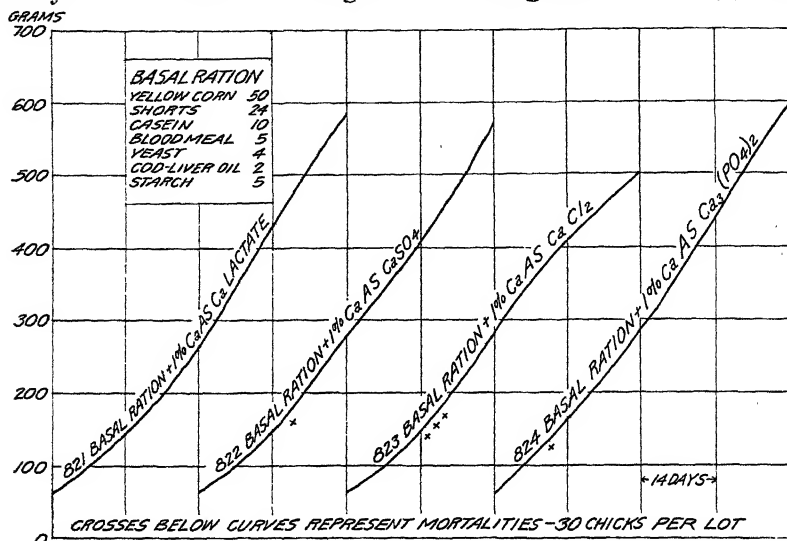


FIGURE 5.—The effect of a 1 per cent calcium supplement upon the growth of chicks, when supplied in the form of various calcium salts

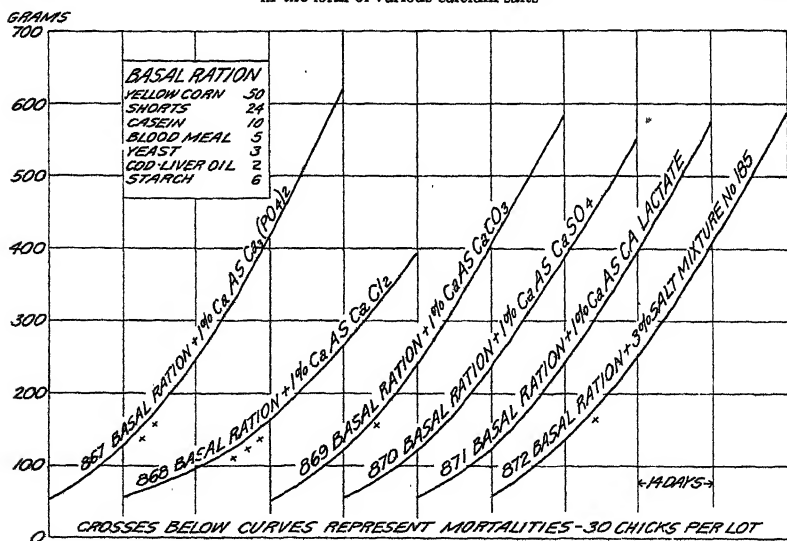


FIGURE 6.—The effect of a calcium supplement upon the growth of chicks, when supplied in the form of various calcium salts, as compared with growth on the basal ration supplemented with a special salt mixture

that a more or less balanced condition of calcium, magnesium, and phosphorus salts of the ration is essential to normal growth and functioning. They found high magnesium content to be a disturbing factor in nutrition. They observed greater tolerance for MgSO_4

than for MgCO_3 when added in equivalent amounts to the same basal ration. Malcolm (4) reports that the ingestion of magnesium salts (MgCl_2) may hinder the deposition of calcium in young animals and cause a loss of calcium from the bodies of older animals. Hart

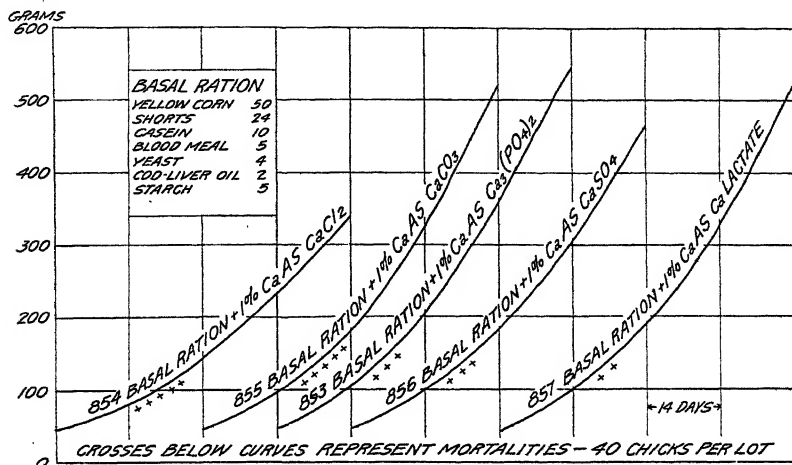


FIGURE 7.—The effect of a 1 per cent calcium supplement upon the growth of chicks, when supplied in the form of various calcium salts

and Steenbock (2) found that with pigs the ingestion of excessive amounts of magnesium salts caused an increased excretion of calcium in the urine.

Figure 8 shows the growth history of three lots of 35 chicks each; one lot receiving no magnesium, one receiving MgCO_3 in quantity

representing 0.5 per cent magnesium, and the third receiving an equivalent amount of MgSO_4 . That there was some disturbance of the mineral metabolism in lot 864 receiving the MgCO_3 was evident from a tendency toward rickets. Seven chicks in this lot were rachitic, whereas the chicks in lot 865 were to all appearances normal.

DISCUSSION

The growth period covered by these experiments extended from the second week to the ninth week, inclusive. The weight increase for

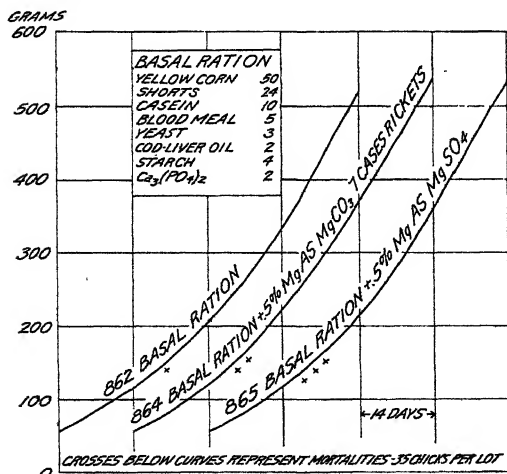


FIGURE 8.—The effect of a 0.5 per cent magnesium supplement, supplied as the carbonate or the sulphate, upon the growth of chicks, as compared with growth on the basal ration containing a tricalcium phosphate supplement

Single-comb White Leghorn chicks on complete rations during this period is normally about 1,000 per cent of the weight at the beginning of the experiment. Growth during this period should reflect quite

accurately the efficiency with which mineral elements are utilized by the growing chick at a time when the inorganic requirements are relatively high.

The utilization of calcium in normal bone growth appears to depend on two factors, (1) its assimilation, and (2) its fixation in the bone cell itself. The action of vitamin D on calcium and phosphorus assimilation is, according to Yoder (9), effective through a lowering of the pH values throughout the intestinal tract. This investigator noted a correlation between lowered pH values and calcium and phosphorus assimilation. Irradiation of rats on a rickets-producing ration likewise resulted in a lowered pH of the intestinal tract, with the distinction, however, that the low pH was observed beyond the duodenum only. This is taken to indicate the excretion of an acid or an acid-producing substance into the small intestine as a result of the action of radiant energy.

In all the experiments for which growth data are here presented the writers have aimed to provide the most favorable conditions for assimilation. As previously noted, a good grade of cod-liver oil was included in the basal ration, and in addition chicks were exposed to direct sunshine. The calcium-phosphorus ratio was not changed when calcium was furnished in each of four forms (not including $\text{Ca}_3(\text{PO}_4)_2$) and the antirachitic factor was provided. Even with these standard conditions, however, some factor influenced the growth rate in the various lots receiving different forms of calcium. The possibility that calcium in certain combinations may result in faulty mineral metabolism in the bone cell itself is to be considered. Jones (3) concludes that the precipitation of bone salts is determined by the balance of ions between the bone-forming cells and their surrounding medium and that such factors as ultra-violet rays, vitamin D, etc., may either directly or by catalysis alter the hydrogen-ion concentration of the bone cells and thus influence the deposition and resorption of bone.

SUMMARY

The growing chick is fortunately able to adjust itself to rather wide ranges of acidity and alkalinity in the diet when vitamin D and ultra-violet radiation are provided. The toleration for acidity and alkalinity is not, however, unlimited, though it probably extends beyond the points which are reached when practical chick rations are fed under farm conditions.

With the particular ration used as a base in these experiments calcium furnished as tricalcium phosphate was more efficiently utilized than was an equivalent amount of calcium furnished as the carbonate, lactate, sulphate, or chloride.

The addition of 0.5 per cent of magnesium, offered as carbonate and sulphate, did not appreciably influence the growth rate of chicks, though rickets resulted from the magnesium carbonate additions.

LITERATURE CITED

- (1) HAAG, J. R., and PALMER, L. S.
1928. THE EFFECT OF VARIATIONS IN THE PROPORTIONS OF CALCIUM, MAGNESIUM, AND PHOSPHORUS CONTAINED IN THE DIET. *Jour. Biol. Chem.* 76: 367-389, illus.
- (2) HART, E. B., and STEENBOCK, H.
1913. THE EFFECT OF A HIGH MAGNESIUM INTAKE ON CALCIUM RETENTION BY SWINE. *Jour. Biol. Chem.* 14: 75-80.

-
- (3) JONES, M. R.
1927. STUDIES ON INORGANIC SALT METABOLISM IN DOGS. ON CERTAIN FACTORS WHICH INFLUENCE THE DEPOSITION AND RESORPTION OF BONE. *Amer. Jour. Physiol.* 79: 694-705, illus.
- (4) MALCOLM J.
1905. ON THE INTER-RELATIONSHIP OF CALCIUM AND MAGNESIUM EXCRETION. *Jour. Physiol.* 32: [183]-190.
- (5) MUSSEHL, F. E., BLISH, M. J., and ACKERSON, C. W.
1926-27. MINERAL METABOLISM OF THE GROWING CHICK. *Poultry Sci.* 6: 239-242, illus.
- (6) SHELLING, D. H., KRAMER, B., and ORENT, E. R.
1928. STUDIES UPON CALCIFICATION IN VITRO. INORGANIC FACTORS DETERMINING CALCIFICATION. *Jour. Biol. Chem.* 77: 157-170.
- (7) SHOHL, A. T., BENNETT, H. B., and WEED, K. W.
1928. RICKETS IN RATS. IV. THE EFFECT OF VARYING THE ACID-BASE CONTENT OF THE DIET. *Jour. Biol. Chem.* 78: 181-190.
- (8) STEENBOCK, H., HART, E. B., SELL, M. T., and JONES, J. H.
1923. THE AVAILABILITY OF CALCIUM SALTS. *Jour. Biol. Chem.* 56: 375-386, illus.
- (9) YODER, L.
1927. EFFECT OF THE ANTIRACHITIC VITAMIN ON THE PHOSPHORUS, CALCIUM, AND PH IN THE INTESTINAL TRACT. *Jour. Biol. Chem.* 74: 321-329, illus.
- (10) ZUCKER, T. F., JOHNSON, W. C., and BARNETT, M.
1923. THE ACID BASE RATIO OF THE DIET IN RICKETS PRODUCTION. *Soc. Expt. Biol. and Med. Proc.* 20: 20-22.



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EFFECT OF DIET ON THE RESISTANCE OF THE ALBINO RAT TO *BACTERIUM ABORTUS*¹

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INTRODUCTION

The albino rat appears to have been used but little in experiments with *Bacterium abortus*, perhaps on account of its comparatively high resistance to this organism.^{2 3} However, since the nutritive requirements of the rat have been studied extensively, it has seemed desirable to ascertain the extent to which the resistance of this animal to *Bact. abortus* is affected by certain dietary deficiencies.

EXPERIMENTAL DATA

The rats used in these experiments were raised in the bureau laboratories from healthy, vigorous, and prolific stock. Mature or nearly mature rats were employed. Unless otherwise stated, the rats were fed a diet fully adequate for excellent growth and reproduction. This diet was made up as follows:

Adequate diet for rats	Per cent
Yellow corn meal.....	25
Graham flour.....	25
Oatmeal.....	24
Dried beef.....	15
Dried bakers' yeast.....	4
Calcium carbonate.....	4
Cod-liver oil.....	2
Sodium chloride.....	1

A single strain of *Bact. abortus* which has been propagated in these laboratories for several years was used in all experiments. This strain is pathogenic for guinea pigs and exhibits typical cultural and agglutinating properties. In general, 4 or 5 day bouillon cultures were used for inoculation and feeding tests, but glycerin-agar cultures were fed in a few experiments.

Rats which had been exposed to *Bact. abortus* were usually killed at the end of from four to five weeks. Six dilutions of the blood serum were used for agglutination tests, viz, 1 to 25, 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000. The internal organs were carefully examined and small quantities of the spleen were transferred to serum-agar slants and spread evenly over the surface. An effort was made to transfer approximately the same quantity of spleen to each tube. The cultures were incubated from six to eight days at 37.5° C., when the colonies of *Bact. abortus* were counted.

¹ Received for publication June 18, 1929; issued February, 1930.

² BURNET, E., and LAGANÈRE, J. L. DE POUVOIR PATHOGÈNE DU *B. MELITENSIS* ET DU *B. ABORTUS* POUR LE RAT ET LA SOURIS. Arch. Inst. Pasteur Tunis 13: 182-191. 1924.

³ HAGAN, W. A. THE SUSCEPTIBILITY OF MICE AND RATS TO INFECTION WITH *BACILLUS ABORTUS*. Jour. Expt. Med. 36: 727-733. 1922.

FEEDING EXPERIMENTS WITH BACTERIUM ABORTUS

EXPERIMENT 1. ADEQUATE DIET

Eighteen rats which had received the previously described adequate diet from birth were fed pure cultures of *Bact. abortus* on several successive days. Four to five weeks later the rats were killed and examined. *Bact. abortus* was isolated from the spleen of only one rat, but agglutination reactions in dilutions ranging from 1 to 25 to 1 to 100 were yielded by the blood serum of eight rats.

EXPERIMENT 2. DIET LOW IN VITAMIN A

Six rats which had been fed a diet low in vitamin A from birth were fed a pure culture of *Bact. abortus* on several successive days. The diet was made up as follows:

Diet low in vitamin A	Per cent
Graham flour.....	36
Oatmeal.....	40
Dried beef.....	15
Dried bakers' yeast.....	4
Calcium carbonate.....	4
Sodium chloride.....	1

Three weeks after being fed the organism the rats were killed and examined. *Bact. abortus* was isolated from the spleens of two rats.

The serum from one of these reacted to the agglutination test in a dilution of 1 to 25, and that from another rat reacted in the same dilution.

EXPERIMENT 3. DIET VERY POOR IN VITAMIN A.

Six rats which had been fed the above-described diet from birth were transferred to a ration very much poorer in vitamin A, as follows:

Diet very poor in vitamin A	Per cent
Casein.....	24
Dried brewers' yeast.....	5
Ash mixture.....	4
Hydrogenated cottonseed oil.....	10
Cassava starch.....	57

The casein was purified from vitamin A by heating in a current of air at from 115 to 130° C. The other constituents have been found to be practically free from this vitamin. This ration is probably poor in vitamin D also, but the rats used appear to have had a very small requirement for this vitamin, apparently because of considerable sunlight which entered the room through the partly opened windows.

After this group of rats had received the foregoing diet for 102 days they were fed a pure culture of *Bact. abortus* on five successive days. Five weeks later they were killed and examined. *Bact. abortus* was isolated from the spleen of only one rat, which also yielded an agglutination reaction in a dilution of 1 to 25. Agglutination reactions were obtained with the blood of two other rats in a dilution of 1 to 50. At the end of the test one rat was in very poor condition but the others were in fair condition. None had ophthalmia.

EXPERIMENT 4. ADEQUATE DIET

Four rats which had received an adequate diet from birth were fed approximately 10 c. c. of the contents of the stomach of an aborted calf infected with *Bact. abortus*. Five weeks later the rats were killed and examined. *Bact. abortus* was isolated from the spleen of one rat, but no agglutination reactions were obtained.

The results of these four experiments indicate that the albino rat is not readily infected with *Bact. abortus* when fed a pure culture of the organism, whether the rats have had an adequate diet or one deficient in vitamin A. Thus, of 34 rats which were used in these feeding tests only five were found to be infected with *Bact. abortus*. This method of exposing rats to *Bact. abortus*, therefore, was not regarded as satisfactory for testing the effects of deficient diets on the resistance of the rat to this organism.

INOCULATION EXPERIMENTS

EXPERIMENT 5. ADEQUATE DIET

Six rats which had been fed an adequate ration were inoculated intra-abdominally with 0.5 c. c. of a bouillon culture of *Bact. abortus* and 37 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of all six rats, the number of colonies on the tubes of serum agar ranging from 1 to 13. All rats afforded a positive agglutination reaction, the titer being 1 to 1,000 or higher. This method of inoculation appeared to be too severe for testing the effects of deficient diets on the resistance of the rat to *Bact. abortus*.

EXPERIMENT 6. ADEQUATE DIET

Six rats which had been on an adequate diet were inoculated subcutaneously with 0.5 c. c. each of a bouillon culture of *Bact. abortus*, and 37 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of three rats, the number of colonies per tube ranging from one to five. The agglutination test was positive in all cases in dilutions of 1 to 100 or 1 to 200, the average being 1 to 150.

EXPERIMENT 7. ADEQUATE DIET

This experiment was conducted to determine the effects of inoculating rats subcutaneously with *Bact. abortus* as regards (1) the duration of the infection in the spleen, and (2) the persistence of agglutinins in the blood. Forty-six rats which had been fed an adequate ration were inoculated subcutaneously with *Bact. abortus* on the same date, and at intervals thereafter groups of three rats were killed and examined, with the results shown in Table 1.

The data in Table 1 indicate roughly the course of the infection in the spleens of rats inoculated subcutaneously with *Bact. abortus*. As judged by cultures taken from the spleens of rats killed at intervals during this experiment, the rats appeared to harbor large numbers of *Bact. abortus* in their spleens on the sixth day after inoculation, but the infection gradually subsided and had apparently disappeared by the forty-sixth day. The spleens of rats examined on the thirty-fifth and forty-second days, respectively, were only slightly infected.

TABLE 1.—Duration of *Bacterium abortus* infection in albino rats following subcutaneous inoculation

Rat No.	Period following inoculation	Agglutination titer	Colonies in duplicate cultures from spleen ^a		Weight of spleen as percentage of body weight	Rat No.	Period following inoculation	Agglutination titer	Colonies in duplicate cultures from spleen ^a		Weight of spleen as percentage of body weight
			A	B					A	B	
	Days		Number	Number	Per cent		Days		Number	Number	Per cent
1.....	6	1-100	400-500	0.530	24.....	42	1-1,000	1	0	.529
2.....	6	1-200	500-600627	25.....	46	1-200	0	0	.518
3.....	6	1-200	500-600725	26.....	46	1-50	0	0	.436
4.....	11	1-200	75-100475	27.....	46	1-200	0	0	.629
5.....	11	1-1,000	75-100535	28.....	56	1-500	0	0	.475
6.....	11	1-200	100-150465	29.....	56	1-1,000	0	0	.372
7.....	16	1-50	100-125	75-100	.420	30.....	56	1-100	0	0	.522
8.....	16	1-200	2	25	.461	31.....	65	(^b)	0	0	.265
9.....	16	1-200	75-100	75-100	.483	32.....	65	1-500	0	0	.324
10.....	21	1-200	50-75	50-75	.563	33.....	65	1-200	0	0	.324
11.....	21	1-100	15-25	15-25	.353	34.....	80	1-200	0	0	.281
12.....	21	1-200	100-150	75-100	.508	35.....	80	1-200	0	0	.298
13.....	24	1-200	21	15	.433	36.....	80	1-100	0	0	.275
14.....	24	1-200	15	6	.508	37.....	100	(^b)	0	0	.244
15.....	24	1-200	11	15	.296	38.....	100	1-200	0	0	.305
16.....	29	1-200	3	0	.502	39.....	100	1-200	0	0	.327
17.....	29	1-50	12	9	.456	40.....	113	1-100	0	0	.297
18.....	29	1-200	1	1	.569	41.....	113	(^b)	0	0	.252
19.....	35	1-1,000	0	0	.421	42.....	113	1-25	0	0	.214
20.....	35	1-200	0	0	.567	43.....	122	(^b)	0	0	.335
21.....	35	1-200	1	0	.421	44.....	122	1-50	0	0	.221
22.....	42	1-200	0	0	.665	45.....	122	1-100	0	0	.299
23.....	42	1-1,000	1	0	.582	46.....	122	(^b)	0	0	.217

^a In numerous instances the number of colonies of *Bact. abortus* on the surface of agar slants could not be counted accurately. In such cases the number was approximated.

^b Negative.

There was considerable variation in the agglutination titer of the blood of rats killed at different intervals. Toward the end of the experiment the titer became lower and the blood of some rats did not react.

The relation between the weight of the spleen and the live weight of the rat indicates a material enlargement of that organ from the sixth to the fifty-sixth days, inclusive, after inoculation with *Bact. abortus*, with a gradual reduction in the size of the spleen from that time until the end of the experiment.

The results of this experiment seemed to indicate that subcutaneous inoculation would be a satisfactory method for exposing rats to *Bact. abortus* in order to test their resistance to this organism as affected by diet. Since rats fed an adequate diet were regularly infected with *Bact. abortus* by this method, and since the infection ran a mild course, it seemed probable that adverse effects of deficient diets would become apparent when infection was accomplished by this means. Experiments with rats fed inadequate diets were therefore carried out.

EXPERIMENT 8. DIETS POOR IN VITAMIN A

Twelve rats which had been fed a diet poor in vitamin A⁴ since birth were inoculated subcutaneously with *Bact. abortus* and 39 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of 3 of the 12, the average number of colonies

⁴ Ration described under experiment 2.

per tube being five. In all rats the agglutination test was positive in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

A control group of 12 rats which had been fed an adequate diet from birth was inoculated at the same time as the preceding group. Thirty-nine days later the rats were killed and examined. *Bact. abortus* was isolated from the spleens of 6 of the 12, the average number of colonies per tube being five. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 644.

Ten rats which had been fed a diet low in vitamin A⁵ from birth were placed on the following diet, which was very deficient in this vitamin.

Diet very poor in vitamin A	Per cent
Caseine (purified).....	24.3
Bakers' yeast.....	10.0
Ash mixture.....	4.0
Hydrogenated cottonseed oil.....	7.0
Wheat-germ oil.....	3.0
Cassava starch.....	51.7

The rats were fed this ration for 28 days, when they were inoculated with *Bact. abortus*. After 31 days more on this ration they were killed and examined. *Bact. abortus* was isolated from the spleens of 9 of the 10. The average number of colonies per tube was four. Serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 100 to 1 to 500, the average being 1 to 370. These rats were in fair condition at the end of the test and showed no signs of ophthalmia.

A control group of nine rats which had received an adequate diet from birth was inoculated at the same time as the preceding group. Thirty-two days later these rats were killed and examined. *Bact. abortus* was found in the spleens of 7 of the 9, the average number of colonies per tube being two. All rats afforded a positive agglutination test in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

The results of these tests indicate no significant difference in the resistance to *Bact. abortus* of rats fed diets deficient in vitamin A as compared with others fed a diet containing an abundance of this vitamin.

EXPERIMENT 9. DIETS POOR IN THE ANTINEURITIC VITAMIN B

Seven rats which had been fed an adequate diet from birth were placed for 38 days on a ration containing only a small quantity of the antineuritic vitamin B. The rats were then inoculated subcutaneously with *Bact. abortus* and kept on this diet for 32 days longer, when they were killed and examined for the organism. The diet was made up as follows:

Diet low in vitamin B	Per cent
Dried beef.....	18.1
Ash mixture.....	4.0
Dried bakers' yeast.....	1.5
Cod-liver oil.....	2.0
Hydrogenated cottonseed oil.....	8.0
Cassava starch.....	66.4

⁵ Ration described under experiment 2.

With the exception of yeast, the constituents of this ration were practically free from vitamin B, the beef having been heated to destroy any of this vitamin normally present. The proportion of yeast in the ration furnished considerably less vitamin B than is required for mature rats, as is indicated by the fact that the rats lost an average of 29 per cent in weight on this diet. After inoculation with *Bact. abortus* the rats were fed this diet for an additional period of 32 days, or a total of 70 days. Most of the animals were in poor condition at the end of this period. *Bact. abortus* was isolated from the spleens of five of the seven rats, the average number of colonies per tube being four. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 50 to 1 to 1,000, the average being 1 to 364.

A second group of 10 rats which had previously received an adequate diet was fed for 11 days a ration containing practically no antineuritic vitamin B. This ration was made up as follows:

Diet very poor in vitamin B	Per cent
Dried beef (autoclaved)-----	19.1
Ash mixture-----	4.0
Cod-liver oil-----	2.0
Hydrogenated cottonseed oil-----	8.0
Cassava starch-----	66.9

The rats were then inoculated subcutaneously with *Bact. abortus* and continued on this diet for 32 days, or a total of 43 days. Two rats died during the test and the others were in very poor condition at the end, having lost 25 per cent in weight. The rats were killed and examined with the result that *Bact. abortus* was isolated from the spleens of seven of the eight rats, the average number of colonies per tube being four. The cultures from the spleen of the other rat were contaminated. Seven of the rats afforded agglutination reactions in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 339.

A third group of rats which had received an adequate diet from birth was fed the same deficient ration as that fed the preceding group. After 11 days on this diet the rats were inoculated subcutaneously with *Bact. abortus* and were continued on the same diet for 28 days, or a total of 39 days, when they were killed and examined. At this time most of the rats were in very poor condition, having lost an average of 25 per cent in weight during the test. *Bact. abortus* was isolated from the spleens of seven of the eight rats used, the average number of colonies per tube being 30. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 50 to 1 to 500, the average being 1 to 331.

As a control, a fourth group of nine rats which had previously received an adequate diet was inoculated subcutaneously with *Bact. abortus* at the same time as Groups 1 and 2 and continued on the same diet for 32 days. The rats were then killed and examined, with the result that *Bact. abortus* was isolated from the spleens of seven of the nine rats, the average number of colonies per tube being two. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

These tests indicate that a deficiency of the antineuritic vitamin B had no significant effect on the resistance of the rat to *Bact. abortus*. Cultures from the spleens of the third group of rats fed a ration de-

ficient in vitamin B showed considerably more colonies of *Bact. abortus* than cultures from two other groups of rats fed a similar diet, or than cultures from control rats fed an adequate diet. However, this difference may have been due to the fact that the third group of rats was killed on the twenty-eighth day after inoculation, whereas the three other groups were killed on the thirty-second day. In Table 1 it will be seen that the time between inoculation and examination of the rats has an important bearing on the number of colonies of *Bact. abortus* in the spleens. Thus, cultures from rats killed on the twenty-ninth day showed more colonies than cultures from rats killed on the thirty-second day.

EXPERIMENT 10. DIET POOR IN VITAMIN E

Eighteen female rats which had previously received an adequate diet were placed on a diet very deficient in vitamin E for a preliminary period of 105 days. This diet, which has proved to be very inadequate for reproduction, was made up as follows:

Diet poor in vitamin E	Per cent
Casein.....	24.3
Ash mixture.....	4.0
Dried bakers' yeast.....	10.0
Lard.....	8.0
Cod-liver oil.....	2.0
Cassava starch.....	51.7

The rats were then inoculated subcutaneously with *Bact. abortus* and continued on the same diet for 31 days longer, or a total period of 136 days. They were then killed and examined for the organism. *Bact. abortus* was isolated from the spleens of 17 of the 18 rats; cultures from the other rat were contaminated. The average number of colonies per tube was 12. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 100 to 1 to 1,000, the average being 1 to 322.

As a control, eight rats which had previously received an adequate diet were inoculated subcutaneously with *Bact. abortus* at the same time as the preceding group. The rats were continued on this diet and 31 days later they were killed and examined, with the following results: *Bact. abortus* was isolated from the spleen of each rat in the control group, the average number of colonies per tube being nine. The serum from each rat reacted to the agglutination test, the dilutions ranging from 1 to 50 to 1 to 500, the average being 1 to 313.

The results of these tests show that the rats fed a diet very poor in vitamin E for 136 days were not materially less resistant to *Bact. abortus* than were control rats fed an adequate diet.

EXPERIMENT 11. DIET POOR IN CALCIUM AND PHOSPHORUS

Fourteen rats which had previously received an adequate diet were fed one very poor in calcium and phosphorus for 73 days. The diet was made up as follows:

Diet low in calcium and phosphorus	Per cent
Graham flour.....	94
Dried beef.....	5
Sodium chloride.....	1

This food mixture contained 0.37 per cent phosphorus but calcium was not determined. According to Sherman,⁶ wheat contains 0.45 per cent calcium, and meat 0.058 gm. calcium per 100 gm. of protein. Since the dried meat in this diet contained 82.7 per cent protein it probably contained approximately 0.048 per cent calcium, or practically the same proportion as was present in the Graham flour. According to McCollum and Simmonds,⁷ wheat is too poor in both calcium and phosphorus to meet the needs of the growing rat. The diet above mentioned contained 15 per cent of a mixture of beef and wheat proteins, which is ample for normal growth in rats. The diet was probably poor in vitamin A.

After the rats had been fed the calcium-phosphorus-deficient diet for 73 days they were inoculated subcutaneously with *Bact. abortus*. The rats were continued on the same diet, and 34 days later, or after a total period of 107 days on this diet, 11 of them were killed and examined for the organism. Five days later the remaining three were killed and examined. The results were as follows: *Bact. abortus* was isolated from the spleens of 7 of the 14, the average number of colonies per tube being eight. The serum from all rats afforded agglutination reactions in dilutions ranging from 1 to 50 to 1 to 1,000, the average being 1 to 525.

As a control, 12 rats, receiving an adequate diet were inoculated subcutaneously with *Bact. abortus* on the same date as the rats fed the calcium-phosphorus-deficient diet. Thirty-nine days later the control rats were killed and examined, with the following results: *Bact. abortus* was isolated from the spleens of 5 of the 12 rats, the average number of colonies per tube being five. All rats afforded agglutination reactions in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 644.

The results of this experiment indicate that the rats fed a diet poor in calcium and phosphorus for 107 days were not materially less resistant to *Bact. abortus* than were rats fed an adequate diet.

A careful post-mortem examination was performed on all the rats used in these experiments. Definite lesions, such as are commonly found in guinea pigs inoculated with *Bact. abortus*, that is, nodular spleens and livers dotted with necrotic foci, were not observed. Enlargement of the spleen, while not pronounced, seemed to occur frequently in infected rats, as determined by a comparison between the weight of that organ and the live weight of the rat.

SUMMARY OF RESULTS

In this paper are reported the results of feeding experiments with albino rats to determine the effects of certain dietary deficiencies on the resistance of these animals to *Bact. abortus*. Rations deficient in vitamin A, the antineuritic vitamin B, vitamin E, and in calcium and phosphorus, respectively, were without significant effect on the resistance of the rat to this organism.

⁶ SHERMAN, H. C. CHEMISTRY OF FOOD AND NUTRITION. Ed. 3, rewritten and enl., p. 590, 593. New York. 1926.

⁷ MCCOLLUM, E. V., and SIMMONDS, N. THE NEWER KNOWLEDGE OF NUTRITION; THE USE OF FOODS FOR THE PRESERVATION OF VITALITY AND HEALTH. Ed. 3, entirely rewritten, p. 129. New York. 1925.

TRANSMISSION OF PULLORUM DISEASE (BACILLARY WHITE DIARRHEA) IN INCUBATORS^{1 2 3}

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HISTORICAL

Since the memorable discovery reported by Rettger⁵ in 1900 that a specific white diarrhea of baby chicks was caused by the organism which he isolated and named *Bacterium pullorum*⁶ extensive fundamental investigations have been conducted with reference to this disease. With the rapid rise in economic importance of the hatchery industry in this country, however, it is evident that much ground yet remains to be covered in pullorum-disease control. The dissemination of the disease among chicks in incubators is a large factor governing the net profit, of the hatcheryman on the one hand, and the livability and vigor of chicks delivered to the poultryman on the other. Indeed, the problem goes further than this, since it also affects to a large extent the perpetuation of the disease from generation to generation.

In 1926 Hinshaw, Upp, and Moore⁷ and Bushnell, Hinshaw, and Payne⁸ reported on the transmission of the disease in incubators. These investigators exposed chicks in an incubator to artificial infection consisting of chick down which had been saturated with *Salmonella pullorum* cultures and dried. The chick mortality from pullorum disease in the infected compartment of the incubator was 59.6 per cent, while in a noninfected compartment exposed to the infected compartment mortality was 24.17 per cent. The controls showed no mortality from this cause.

In 1927 Hinshaw⁹ called attention to the need of further investigation of the subject and suggested that the following experimental factors be given attention:

- (1) The use of various types of incubators.
- (2) Dissemination of the disease by naturally infected chicks from eggs of hens reacting to the agglutination test for pullorum disease.

¹ Received for publication July 6, 1929; issued February, 1930.

² The term "bacillary white diarrhea" and its abbreviation "b. w. d.," have acquired considerable standing through long use in the poultry industry, as well as in contemporary research. It has been found convenient to employ the latter designation in portions of this paper. For further information see page 210.

³ At the suggestion of the Pennsylvania State Department of Agriculture, Rettger in 1923 proposed to substitute the name "pullorum disease" to denote the infection in either young or adult stock. It is so used in this text. See the following publication: RETTGER, L. F., THE NEED OF ACCEPTED SCIENTIFIC STANDARDS AND RIGID ADHERENCE TO THEM IN PULLORUM DISEASE CONTROL. Jour. Amer. Vet. Med. Assoc. (n. s. 27) 74: 453-461. 1929.

⁴ This paper reports work conducted under the direction of the committee on b. w. d. research, of the Bureau of Animal Industry, consisting of M. Dorset, chief, Biochemic Division, chairman; M. A. Jull, senior poultry husbandman, Animal Husbandry Division; and Hubert Bunyea, associate veterinarian, Pathological Division.

⁵ RETTGER, L. F. SEPTICÆMIA AMONG YOUNG CHICKENS. N. Y. Med. Jour. 71: 803-805, illus. 1900.

⁶ In conformity with the latest classification, the organism is now commonly designated as *Salmonella pullorum*.

⁷ HINSHAW, W. R., UPP, C. W., and MOORE, J. M. STUDIES IN TRANSMISSION OF BACILLARY WHITE DIARRHEA IN INCUBATORS. Jour. Amer. Vet. Med. Assoc. (n. s. 21) 68: 631-641. 1926.

⁸ BUSHNELL, L. D., HINSHAW, W. R., and PAYNE, L. F. BACILLARY WHITE DIARRHEA IN FOWL. Kans. Agr. Expt. Sta. Tech. Bul. 21: 46-49. 1926.

⁹ HINSHAW, W. R. THE INCUBATOR AS A MEANS OF TRANSMITTING BACILLARY WHITE DIARRHEA. World's Poultry Cong. Proc. (1927) 3: 372-374. [1928]

(3) The use, as controls, of pullorum-disease-free chicks, from hens which have passed the agglutination test for the disease.

(4) Bacteriological examination of dead chicks and embryos to determine the presence or absence of pullorum disease.

(5) Maintenance of experimental chicks under isolation for 14 days for observation and determination of pullorum infection.

In 1928 Hinshaw, Scott, and Payne¹⁰ reported on further studies in dissemination of pullorum disease in incubators. In each of eight hatches completed in a forced-draught incubator, they placed a tray of eggs from infected hens in one end of the machine, and a tray of normal eggs adjacent thereto. A third tray of normal eggs, placed in the opposite end of the same incubator, and a fourth tray of normal eggs in a separate machine were used as controls. The average mortality from pullorum disease in the several trays was as follows:

Chicks from eggs of reacting hens.....	per cent..	30. 01
Chicks from adjacent tray (normal exposed).....	do.....	36. 75
Chicks from opposite tray (normal exposed).....	do.....	18. 59
Chicks from control incubator (unexposed).....	do.....	0. 0

FORMULATION OF PROJECT

In recognition of the paramount importance of control of pullorum disease to the poultry industry, the National Poultry Council through Roy E. Waite, of the University of Maryland, in 1927 entered into a cooperative agreement with the Bureau of Animal Industry to furnish funds to inaugurate a study of the disease from various angles of practical interest to the industry.

A committee on b. w. d. research, consisting of M. Dorset, chairman, M. A. Jull, and Hubert Bunyea, was appointed by John R. Mohler, chief of the bureau, to act in conjunction with a committee from the National Poultry Council to perfect the plans for the investigation and to direct the course of its execution.

The opinion of the joint committee was that the most urgent problem to be investigated was the transmission of pullorum disease in various types of incubators. It was agreed that for this investigation it would be necessary to provide two kinds of eggs for hatching—(1) eggs from hens known to be free from pullorum infection and (2) eggs from hens known to harbor that infection.

Arrangements were made by E. W. Sheets, chief of the animal husbandry division, to supply disease-free eggs from Rhode Island Red hens at the United States Animal Husbandry Experiment Farm, Beltsville, Md. These hens had been tested once for pullorum disease by the agglutination method, and all reactors had been carefully removed from the flock.

For a dependable supply of pullorum-infected eggs, it was decided to purchase a number of reacting hens from various sources and to maintain them at the bureau's experiment station at Bethesda, Md. About 175 hens, including Rhode Island Reds, Barred Plymouth Rocks, and a few birds of other heavy breeds were obtained for this purpose, through the cooperation of E. M. Pickens, of the College of Agriculture, University of Maryland, and William Moore, State veterinarian, Raleigh, N. C. These birds were retested upon their arrival at the experiment station, and only the strong reactors were

¹⁰ HINSHAW, W. R., SCOTT, H. M., and PAYNE, I. F. FURTHER STUDIES ON DISSEMINATION OF SALMONELLA PULLORUM INFECTION IN INCUBATORS. Jour. Amer. Vet. Med. Assoc. (n. s. 25) 72: 559-610, illus. 1928.

retained for the use of the experiment. The flock thus assembled was divided into suitable units for breeding, given proper quarters, and mated with vigorous young male birds.

EXPERIMENTAL BUILDINGS AND EQUIPMENT

Through the cooperation of the late E. C. Schroeder, former superintendent of the experiment station, and that of his successor, W. E. Cotton, ample facilities in the form of buildings and suitably fenced grounds were made available at the station for the project. Additional buildings at the station, as well as various kinds of laboratory

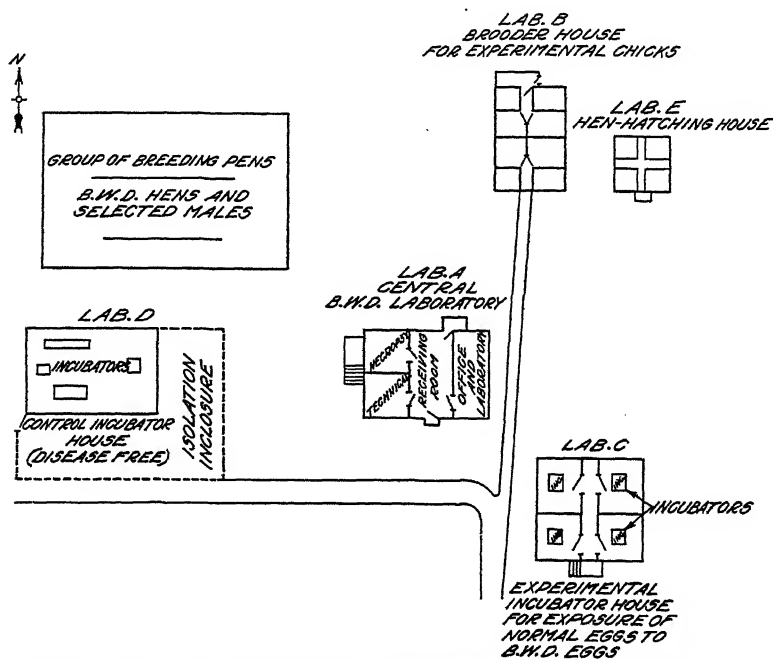


FIGURE 1.—Location and ground plans of buildings used for pullorum-disease research at the experiment station, Bethesda, Md.

and office equipment, were also lent to the project through the cooperation of John S. Buckley, chief of the pathological division. (Fig. 1.)

The buildings occupied by this experiment (fig. 2) consisted of laboratories A, B, C, D, and E, and nine poultry houses and yards. Laboratory A contained the office and bacteriological and pathological laboratory. Laboratory B was the experimental brooder house. Laboratories C and D were the incubator houses, and laboratory E was used for experiments in which hens were used for hatching. Laboratory E was used also for miscellaneous purposes.

Laboratory C was a concrete structure one story high. The floor space was divided into a central hallway running from the front to the back, and four rooms (two on each side), provided with tight, painted walls and seamless waterproof floors, to permit of absolute cleanliness and efficient disinfection of the rooms and their contents.

Each room and the hall were equipped with running water and electricity. The building was uniformly heated by steam heat. There were no communicating doors between rooms. Laboratory C housed the incubators in which the b. w. d. transmission experiments were conducted.

Four different makes of egg incubators were placed in this building, one in each room. These machines will hereinafter be designated

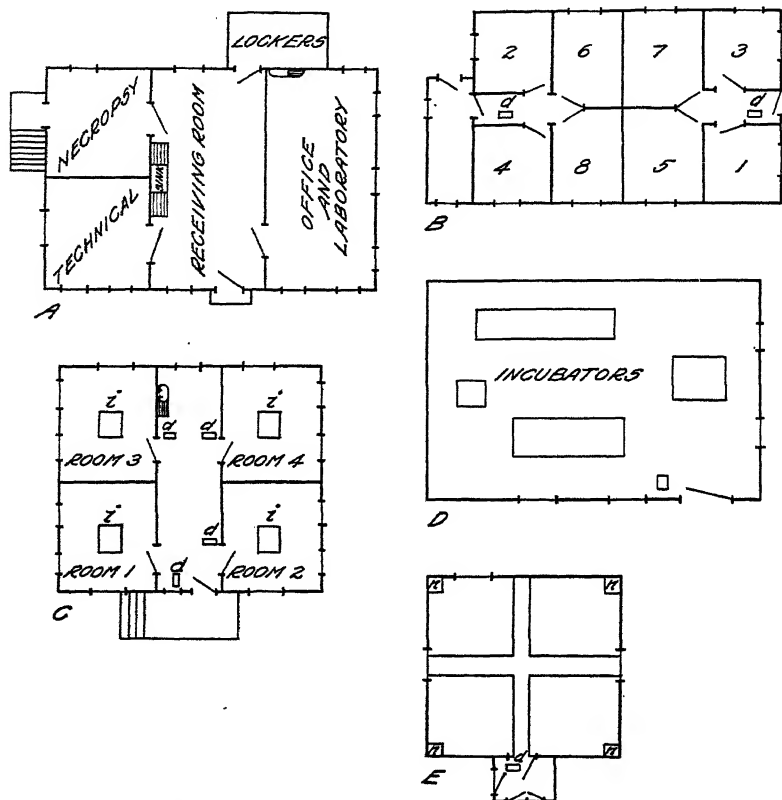


FIGURE 2.—Detailed ground plans of principal experimental buildings used for pullorum-disease research at the experiment station, Bethesda, Md.: A, Laboratory A, containing central b. w. d. office and laboratory of pathology and bacteriology; B, laboratory B, brooder house for experimental chicks, containing eight isolated brooder rooms with disinfectant pans (d); C, laboratory C, experimental incubator house for exposure of normal b. w. d. eggs with incubator (i) in each room and disinfectant pans (d) at doors; D, laboratory D, control incubator house for incubation of normal eggs under disease-free conditions; E, laboratory E, containing four isolated brooding pens for hen hatching and brooding experiments with nests (n) and disinfectant pan (d)

respectively as incubators 1, 2, 3, and 4. Incubators 1 and 4 were of the agitated-air type, while 2 and 3 were of the still-air type.

Laboratory D was a frame building about 20 by 30 feet, metal sealed inside, with concrete floor. The building was equipped with electricity and running water. In the center of the ceiling was a trapdoor about 3 by 5 feet opening into the attic. A large ventilator in the roof directly over this trapdoor furnished ventilation for the building.

This building was located about 200 feet from laboratory C, and housed the incubators used for hatching the control eggs. In this building also there were four incubators, one of each of the makes in laboratory C. The incubators in laboratory D were used as control incubators to hatch a portion of the disease-free eggs, in order to establish more fully their freedom from pullorum infection.

Before beginning a hatch the floor of the incubator room was disinfected with a 5 per cent saponified cresol solution. The room was then fumigated with formaldehyde gas, generated by mixing 16½ ounces of potassium permanganate with 20 ounces of formalin per 1,000 cubic feet of air space. All openings were closed, the doors being calked with cotton. The rooms thus treated were left closed for a period of more than 18 hours.

The brooder house was a single-story, frame building, 18 by 24 feet, plastered inside and divided across the middle by a solid partition of heavy wall board. Each half of the building was further divided into four rooms of approximately equal proportions, each having a door opening into a common hallway. All joints in the partitions were packed with felt to prevent passage of germ-laden dust from one room to another. Each room had one screened window (except room 3, which had two windows) fitted with a ventilator draft board at the bottom, and a dark, adjustable window shade. An electric outlet and a hot-water radiator were supplied for each room. The doors swung in and were hung on spring hinges to close automatically. They were accurately fitted so as practically to exclude all air, except what was allowed to permeate through the muslin panel in the upper half of the doors.

The rooms at one end of the building were used to house the normal chicks and those in the opposite end to house the b. w. d. chicks. As there was no communication between these two halves of the building, it was necessary to go out around the building to get from one section to the other. This was a sanitary precaution to prevent the transmission of infection from one part of the building to the other. For the same purpose the attendant wore rubber gloves, sleeves, apron, and boots which were disinfected upon entering the building and before entering each brooder room. Visitors were strictly excluded from these rooms during the experiments.

The brooder equipment (fig. 3) for each room was mounted on a painted wooden table and consisted of an electric brooder resting on a ¾-inch-mesh wire floor within a brooder pan 3 by 5 feet and 3 inches high. The wire cloth floor was 1 inch above the bottom of the brooder pan to allow the droppings of the chicks to fall through beyond their reach. On the sides and one end of the brooder pan were vertical, circular perforations, 1 inch in diameter, outside of which food and water receptacles were placed so as to be constantly accessible to and at the same time prevent contamination of the food and water by the chicks. A 12-inch wire inclosure surmounted the pan on all sides.

Before introducing a brood of chicks into any of these brooders, the room was thoroughly disinfected by spraying with a 5 per cent solution of saponified cresol, and all the equipment in the room was either sprayed or dipped in a like solution.

DEFINITION OF TERMS

It is necessary to explain the meanings of certain abbreviations that appear later in tables and diagrams:

B. w. d. (bacillary white diarrhea) designates fowls that are infected with bacillary white diarrhea (pullorum-disease) infection, or the eggs or chicks from such fowls.

N. e. (normal exposed) designates the eggs or chicks from normal hens (hens free from pullorum disease), such eggs or chicks having been exposed in an incubator to the presence of b. w. d. eggs or chicks.

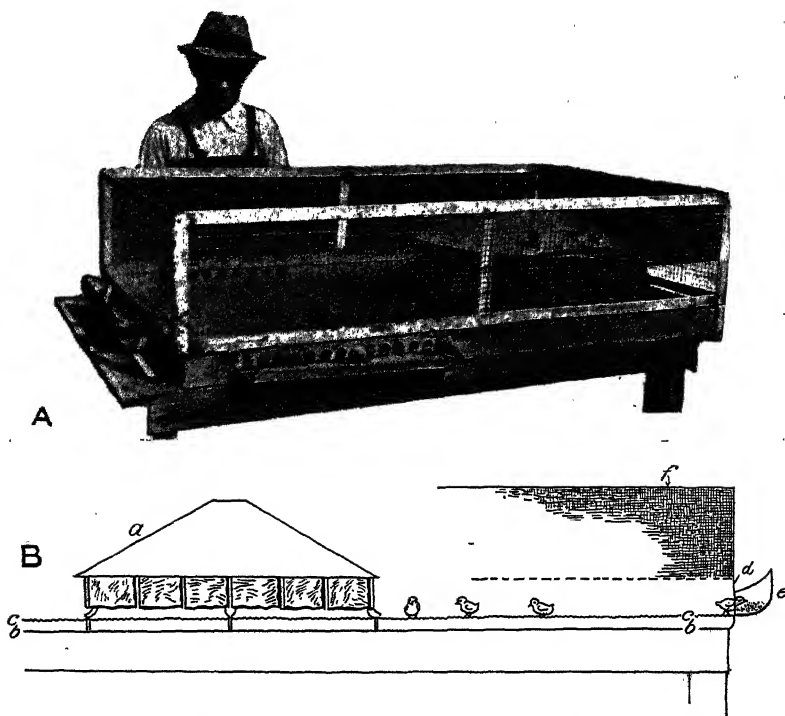


FIGURE 3.—Special brooding equipment used for pullorum-disease experiments: *a*, electric canopy brooder; *b b*, bottom of brooder pan; *c c*, wire-cloth floor; *d*, side of brooder pan perforated; *e*, arrangement for supplying food and water from outside brooder; *f*, 12-inch wire-cloth fence around brooder

N. c. (normal control) designates the eggs or chicks from the same normal flock as the n. e. eggs or chicks, but which have not been exposed to the presence of b. w. d. eggs or chicks.

INCUBATION EXPERIMENTS

Incubators in laboratory C were set with normal and with b. w. d. eggs as is shown in Table 1.

TABLE 1.—*Positions of infected and noninfected eggs in incubators*

Date set	Hatch	Incubator	Positions of infected and noninfected eggs in hatching trays
1928			
Feb. 17.....	A	1	At opposite ends (cabinet-type incubator).
Feb. 27.....	A	2	In adjoining compartments (sectional-type incubator).
Feb. 17.....	A	3	In same compartment; tray partitioned by mesh wire.
Do.....	A	4	N. e. eggs in upper tray directly over b. w. d. eggs.
Mar. 20.....	B	1	At opposite ends, same as hatch A 1.
Feb. 28.....	B	2	In adjoining compartments, same as hatch A 2.
Apr. 5.....	B	3	Same as hatch A 3.
Mar. 13.....	B	4	Same as hatch A 4.
Apr. 16.....	C	1	At opposite ends, same as hatch A 1.
Apr. 23.....	C	2	In adjoining compartments, same as hatch A 2.
Apr. 30.....	C	3	Same as hatch A 3.
May 7.....	C	4	Same as hatch A 4.
May 14.....	D	1	B. w. d. eggs in upper tray directly over n. e. eggs.
May 22.....	D	2	In adjoining compartments, same as hatch A 2.
May 28.....	D	3	Same as hatch A 3.
June 4.....	D	4	Same as hatch A 4.
June 11.....	E	1	Same as hatch D 1.
June 18.....	E	2	In adjoining compartments, same as hatch A 2.
June 25.....	E	3	Same as hatch A 3.
July 2.....	E	4	B. w. d. eggs in upper tray directly over n. e. eggs.
July 13.....	F	1	Same as hatch D.
Aug. 6.....	G	4	Do.
Aug. 20.....	H	4	Same as hatch E.
Sept. 14.....	I	4	Do.
Oct. 1.....	K	1	Same as hatch D.
Oct. 23.....	L	4	Same as hatch E.

Some of the b. w. d. eggs used for hatch A in incubators Nos. 1, 3, and 4 were as much as 2 weeks old, as the flock was just coming into production, and the supply of eggs was temporarily limited. None of the eggs set in incubator No. 2 were more than 6 days old.

The eggs in all machines were turned at regular intervals each day, and temperatures and moisture were regulated according to the instructions accompanying each machine.

The room temperatures and incubator temperatures were recorded at least twice each day. These records are available but have not been made a part of this report.

The method of procedure here outlined and the detailed description of the equipment will not be repeated for each experimental hatch, as they were essentially alike for all hatches unless otherwise specifically stated.

BROODING OF CHICKS

After the close of the 21-day period the newly hatched chicks were left in the hatching trays for about 24 hours and were banded with colored celluloid bands for subsequent identification. They were then placed in new, clean chick boxes and taken to their respective brooder rooms in laboratory B, as follows:

Rooms 1, 3, 5, and 7, in the south end of the brooder house, were used to quarter the b. w. d. chicks from the various incubators. Rooms 2 and 4 were used to house the n. e. chicks from the different incubators, while rooms 6 and 8 were used exclusively for the n. c. chicks from the different machines. (Fig. 2, B.) Of course only one brood at a time was allowed in a room, and all rooms were thoroughly disinfected between broods.

After they were 48 hours old, and for the remainder of the 14-day experimental period, the chicks were fed exclusively on a starting mash composed of the following ingredients: Corn meal, fine oat-meal, flour middlings, mineral supplement, meat and bone meal, flake buttermilk, and cod-liver meal. This feed was guaranteed to

contain 14 per cent protein, 4 per cent fat, 3 per cent fiber, and 58 per cent carbohydrates.

The chicks were kept under observation for two weeks. The temperatures of the brooders and rooms were carefully regulated and noted at regular intervals. These records are available but are not included in this report.

All chicks that died within two weeks were autopsied, and cultures made from the liver, lung, and unabsorbed yolk. Any organisms recovered in this way were subjected to further study and tested on various culture media for the characteristics of *Salmonella pullorum*. The following criteria were regarded as sufficient to establish the identity of that organism: Origin, chicken; Gram staining, negative; motility, negative; reaction on litmus milk, slightly acid at 3 days, possibly alkaline in 12 days.

Carbohydrate reactions: Dextrose, acid, with or without gas; lactose, no acid, no gas; saccharose, no acid, no gas; and dulcitol, no acid.

A number of representative cultures of *Salmonella pullorum* recovered from each hatch were also tested in beef-extract broths containing 1 per cent, respectively, of maltose, mannite, and levulose, and were examined for motility and Gram-staining qualities. The cultures were also tested by the agglutination method against hyper-immune *S. pullorum* antiserum.

TABLE 2.—Results of normal control hatches of noninfected eggs, Beltsville, Md.¹

Incubator	Eggs set	Fertile eggs	Dead embryos and dead in shell	Chicks hatched
No. 1.....	Number 96	Number 74	Number 16	Number 58
No. 3.....	96	74	14	60
No. 4.....	96	79	6	73

¹ Owing to the distance between the two experiment stations—Bethesda and Beltsville—and lack of regular transportation facilities, the dead chicks which were sent to the former from the latter place were too far decomposed for bacteriological study when received.

Table 2 gives the results of control hatches in incubators of similar types operated at the United States Animal Husbandry Experiment Farm, about 23 miles from the station at Bethesda. The eggs used in the incubators at Beltsville were of the same age and origin as the normal exposed eggs incubated at Bethesda.

After two hatches had been run in each machine, the control incubators, Nos. 1, 3, and 4, were brought from the Beltsville farm and installed in laboratory D at the Bethesda station. A control incubator of the same make and model as No. 2 was subsequently placed in laboratory D. The new arrangement removed several objectionable features of the former arrangement. As the normal experimental eggs for both the test and the control incubators were taken by automobile from Beltsville to Bethesda any inequality of hatchability otherwise attributable to damage in transit was removed. The chicks that died after being hatched in the control incubators were available for autopsy and bacteriological study while in a fresh condition. In this way the valuable information which otherwise would be lost was conserved.

TABLE 3.—Results of hatch A

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	Number	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
N. e.	240	198	97	49	101	51	42	42	35	83
B. w. d.	192	173	66	38	107	62	78	73	65	83

INCUBATOR NO. 2

N. e.	150	139	22	16	117	84	16	14	6	38
B. w. d.	144	123	45	37	78	63	58	74	54	93

INCUBATOR NO. 3

N. e.	72	61	39	64	22	36	7	32	6	86
B. w. d.	72	58	31	53	27	47	17	63	12	71

INCUBATOR NO. 4

N. e.	280	245	115	47	130	53	32	25	14	44
B. w. d.	196	172	54	31	118	69	44	37	30	68

TABLE 4.—Results of hatch B

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	Number	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
N. e.	192	173	29	17	144	83	76	53	70	92
B. w. d.	192	151	38	25	113	75	60	80	86	96

INCUBATOR NO. 2

N. e.	83	62	41	66	21	34	15	71	6	40
B. w. d.	83	67	38	57	29	43	21	72	20	95

INCUBATOR NO. 3

N. e.	72	60	16	27	44	73	19	43	17	89
B. w. d.	72	60	18	30	42	70	26	62	23	88
N. c.	72	60	30	50	30	50	4	13	0	0

INCUBATOR NO. 4

N. e.	168	154	63	41	91	59	18	20	15	83
B. w. d.	168	143	62	43	81	57	30	37	29	97

TABLE 5.—Results of hatch C

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	Number	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
N. e.-----	96	78	10	13	68	87	8	12	5	63
B. w. d.-----	96	75	21	28	54	72	37	69	33	89
N. c.-----	96	78	16	21	62	79	3	5	0	0

INCUBATOR NO. 2

N. e.-----	84	64	19	30	45	70	2	4	0	0
B. w. d.-----	84	62	23	37	39	63	27	69	24	89

INCUBATOR NO. 3

N. e.-----	72	60	18	30	42	70	3	7	1	33
B. w. d.-----	72	63	28	44	35	56	17	49	16	94
N. c.-----	72	64	18	28	46	72	0	0	0	0

INCUBATOR NO. 4

N. e.-----	96	78	14	18	64	82	2	3	0	0
B. w. d.-----	96	77	23	30	54	70	6	11	4	67
N. c.-----	96	88	13	15	73	85	2	3	0	0

TABLE 6.—Results of hatch D

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	Number	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
N. e.-----	96	87	17	20	70	80	18	26	16	89
B. w. d.-----	96	76	23	30	53	70	40	75	36	90
N. c.-----	96	84	22	26	62	74	5	8	0	0

INCUBATOR NO. 2

N. e.-----	84	72	25	35	47	65	3	6	0	0
B. w. d.-----	84	68	18	26	50	74	30	60	27	90
N. c.-----	84	62	16	26	46	74	7	15	0	0

INCUBATOR NO. 3

N. e.-----	72	62	23	37	39	63	0	0	0	0
B. w. d.-----	72	62	39	63	23	37	11	48	6	56
N. c.-----	72	62	16	26	46	74	5	11	0	0

INCUBATOR NO. 4

N. e.-----	96	76	20	26	56	74	2	4	0	0
B. w. d.-----	96	66	23	35	43	65	10	23	8	80
N. c.-----	96	82	27	33	55	67	4	7	0	0

TABLE 7.—Results of hatch E

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	Number	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
N. e.	72	57	7	12	50	88	18	36	16	89
B. w. d.	72	52	17	33	35	67	26	74	26	100
N. e.	66	52	14	27	38	73	4	11	0	0

INCUBATOR NO. 2

N. e.	72	64	25	39	39	61	4	10	0	0
B. w. d.	72	52	33	63	19	37	10	53	9	90
N. e.	62	51	13	25	38	75	3	8	0	0

INCUBATOR NO. 3

N. e.	72	57	35	61	22	39	7	32	1	14
B. w. d.	72	50	40	80	10	20	5	50	5	100
N. e.	56	44	15	34	29	66	7	24	0	0

INCUBATOR NO. 4

N. e.	72	53	24	45	29	55	2	7	2	100
B. w. d.	72	56	22	39	34	61	14	41	9	64
N. e.	69	44	25	57	19	43	2	11	0	0

Tables 3 to 7 constitute a record of all incubation experiments conducted at the Bethesda station in connection with this investigation.

In order to make a comparative test of the dissemination of pullorum disease in hen-hatched chicks a series of hatches was begun at this juncture to develop simultaneously with certain incubator hatches. Each group of settings allotted to hens contained two or three mixed settings and one normal setting. The mixed settings each consisted of 8 b. w. d. eggs and 8 n. e. eggs.

The building used for these tests (fig. 2, E) was of one room with a single doorway opening into an exterior vestibule. The room contained four brooder pens separated from one another by 16-inch alleys. The inclosures were boarded up 1 foot from the floor, and above that poultry wire was stretched to a sufficient height to prevent the birds from leaving their own inclosures. The hatching nests were located in the extreme corners of the room, one in each inclosure. This building and its equipment were thoroughly disinfected before each series of hatches. Tables 8 and 9 show the result of these experiments.

TABLE 8.—Results of incubator hatches of *n. e.*, *b. w. d.*, and *n. c.* eggs, to be compared with results of hen-hatched eggs recorded in Table 9

N. E.

Hatch	Eggs		Dead embryos and dead in shell		Chicks					
	Set	Fertile			Hatched		Died		Dead from which <i>Salmonella pullorum</i> was recovered	
	Number	Number	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
F.....	48	34	2	6	32	94	3	9	1	33
G.....	72	59	18	31	41	69	13	32	11	85
H.....	72	54	7	13	47	87	4	9	0	0
I.....	172									
K.....	72	55	8	15	47	85	9	19	5	56
L.....	72	52	11	21	41	79	1	2	0	0
Total or average...	408	254	46	18	208	82	30	14	17	57

B. W. D.

F.....	72	38	12	32	26	68	17	65	15	88
G.....	72	60	20	33	40	67	12	30	11	92
H.....	72	55	19	35	36	65	13	36	11	85
I.....	172									
K.....	72	57	8	14	49	86	10	20	7	70
L.....	72	55	8	15	47	85	2	4	0	0
Total or average...	432	265	67	25	108	75	54	27	44	81

N. C.

F.....	120	89	14	16	75	84	2	3	0	0
G.....	70	53	20	38	33	62	1	3	0	0
H.....	64	50	5	10	45	90	2	4	0	0
I.....	108	87	24	28	63	72	0	0	0	0
K.....	120	87	25	29	62	71	4	6	0	0
L.....	155	119	29	24	90	76	11	12	0	0
Total or average...	637	485	117	24	308	76	20	5	0	0

¹ None hatched.TABLE 9.—Results of hen hatches of *n. e.*, *b. w. d.*, and *n. c.* eggs, to be compared with results of incubator-hatched eggs recorded in Table 8

N. E.

Hatch	Eggs			Dead embryos and dead in shell		Chicks					
	Set	Fertile	Broken			Hatched ¹		Died		Dead from which <i>Salmonella pullorum</i> was recovered	
	No.	No.	No.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
F.....	24	22	6	3	19	13	81	1	8	1	100
G.....	24	24	4	5	25	15	75	2	13	1	50
H.....	24	22	3	7	37	12	63	0	0	0	0
I.....	24	20	1	6	32	13	68	1	8	1	100
K.....	24	21	5	4	25	12	75	3	25	1	33
Total or average...	120	109	19	25	28	65	72	7	11	4	57

¹ Hatchability is figured on number of eggs set less broken eggs and infertile eggs.

TABLE 9.—Results of hen hatches of n. e., b. w. d., and n. c. eggs, to be compared with results of incubator-hatched eggs recorded in Table 8—Continued

B. W. D.

Hatch	Eggs			Dead embryos and dead in shell		Chicks					
	Set	Fertile	Broken			Hatched		Died		Dead from which <i>Salmonella pullorum</i> was recovered	
	No.	No.	No.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
F.....	24	18	2	7	44	9	56	2	22	2	100
G.....	24	21	3	6	33	12	67	0	0	0	0
H.....	24	20	5	5	33	10	67	2	20	1	50
I.....	24	21	3	9	50	9	50	0	0	0	0
K.....	24	21	6	5	33	10	67	0	0	0	0
Total or average.....	120	101	19	32	39	50	61	4	8	3	75

N. C.

F.....	16	16	2	5	36	9	64	0	0	0	0
G.....	16	16	5	4	36	7	64	0	0	0	0
H.....	16	12	2	1	10	9	90	0	0	0	0
I.....	16	12	0	1	8	11	92	0	0	0	0
K.....	16	16	9	4	57	3	43	3	100	0	0
Total or average.....	80	72	18	15	28	39	72	3	8	0	0

DISCUSSION

Tables 3 to 7 give in detail the data obtained from hatches A to E, inclusive.

Beginning with hatch B, incubator 3, the results of incubation of the control eggs are incorporated in the tabulations for that and subsequent respective hatches, except incubator 2, hatch C, for which a control incubator was not available.

It will be noted from Tables 8 and 9 that among the n. e. chicks the hatchability in incubators was 82 per cent, and under hens 72 per cent, while the mortality was 14 per cent as compared with 11 per cent for hen-hatched chicks. In both groups *Salmonella pullorum* was recovered in 57 per cent of chicks dying within 14 days.

Among the b. w. d. chicks, hatchability in incubators was 75 per cent and under hens 61 per cent. Mortality of b. w. d. chicks in incubators was 27 per cent and under hens 8 per cent. In incubator-hatched chicks *Salmonella pullorum* was recovered in 81 per cent of chicks dying within 14 days, while in hen-hatched chicks recovery of the organism occurred in 75 per cent of cases.

Among the normal controls, no cases of *Salmonella pullorum* developed either in the incubator-hatched or in the hen-hatched chicks. This is considered the more significant in view of the fact that all the hens used in these hatching experiments were reactors to the agglutination test for *S. pullorum* infection and were taken from among the flock used to supply the infected eggs.

In Table 10 there is presented a summary of the results obtained from hatches A to H in agitated-air incubators and from hatches A to E in still-air incubators. It was originally intended that hatches F, G, and H in agitated-air incubators should be part of another

experiment, rather than being included in the experiments in incubator transmission of pullorum disease. However, inasmuch as these carefully controlled data were available, they were included in this report. This explains the three additional hatches taken from agitated-air incubators.

TABLE 10.—Comparative results of hatches carried on in agitated-air and still-air incubators

HATCHES A, B, C, D, E, F, G, H—AGITATED-AIR INCUBATORS								
Eggs, kind	Eggs set	Fertile eggs	Chicks					
			Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	Number	Number	Per cent	Number	Per cent	Number	Per cent
N. e.-----	1,600	1,346	923	69	238	26	185	78
B. w. d.-----	1,492	1,194	794	66	417	53	363	87
N. c.-----	773	618	463	75	25	5	0	0

HATCHES A, B, C, D, E—STILL-AIR INCUBATORS								
	Number	Number	Number	Per cent	Number	Per cent	Number	Per cent
N. e.-----	833	701	438	62	76	17	37	49
B. w. d.-----	827	665	352	53	222	63	196	88
N. c.-----	418	343	235	69	26	11	0	0

Figures 4, 5, and 6 present in graphic form the average percentages of hatchability, mortality, and transmission of *Salmonella pullorum*

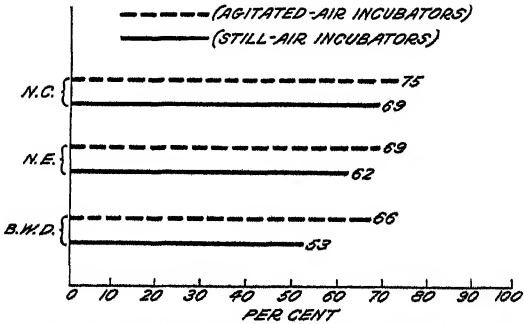


FIGURE 4.—Comparative percentages of hatchability of n. c., n. e., and b. w. d. infected eggs in agitated-air and in still-air incubators

infection, respectively, for hatches A to H in agitated-air incubators and hatches A to E in still-air incubators. While it is possible that under other conditions the results reported in this paper might not be precisely duplicated, the writers feel that their experience as here reported, together with the results reported by Hinshaw and others is sufficient to show that pullorum infection will spread in incubator. It is a dangerous practice to set normal eggs in the same incubator with infected eggs, regardless of the type of incubator used.

SUMMARY AND CONCLUSIONS

Pullorum-disease infection was transmitted from diseased chicks to healthy chicks by exposure in an incubator for 18 to 24 hours from the time of hatching, without actual contact between the chicks.

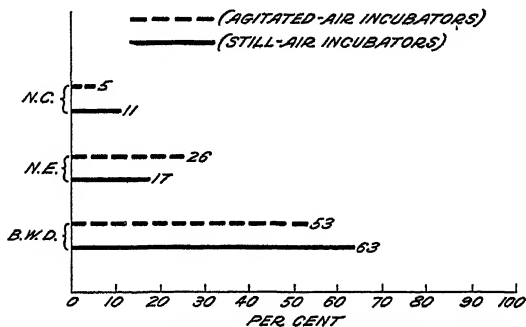


FIGURE 5.—Comparative percentages of mortality of chicks hatched from n. c., n. e., and b. w. d. infected eggs in agitated-air and in still-air incubators

A large percentage of chicks so exposed succumbed to the disease even under the most favorable subsequent brooding conditions.

There appeared to be a seasonal variation in the death rate due to this disease, the losses being greatest during the height of the laying season.

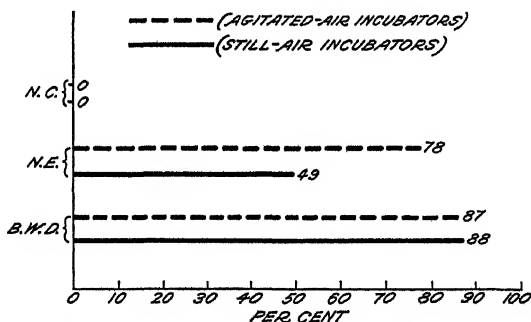


FIGURE 6.—Comparative percentages of transmission of pullorum disease in chicks hatched from n. c., n. e., and b. w. d. infected eggs in agitated-air and in still-air incubators

Hatchability of eggs in incubators was higher than under hens, but livability of hen-hatched chicks surpassed that of incubator chicks. The transmission of pullorum disease among incubator-hatched chicks was about the same as among hen-hatched chicks.



INHERITANCE OF THE SECOND FACTOR FOR RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN HUSSAR WHEAT¹

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INTRODUCTION

In an earlier publication (2)³ the writer presented data which indicated that Hussar wheat possesses two factors for resistance to bunt, *Tilletia tritici* (Bjerk.) Wint. One factor was shown to be the same as the dominant factor present in the Martin variety. The exact effect of the second factor could not be stated definitely at that time because there was some conflict in the evidence. The F₂ data from the cross between Hussar and Hard Federation indicated that the second factor for resistance allowed bunt to develop on about one-half of the heterozygous plants, but the F₃ data suggested that this factor was completely dominant like the factor present in Martin.

In the former paper it was pointed out that the effect of the second factor could be determined by isolating selections possessing the second factor but lacking the factor common to Martin and Hussar. Such a selection has been isolated, and data covering the inheritance of the second factor are now available.

REVIEW OF THE LITERATURE

Much of the literature pertinent to the inheritance of resistance to bunt was reviewed in the earlier publication mentioned above (2). Gaines (4) in 1925 described his extensive investigations on bunt resistance. He grouped varieties into four classes; namely, susceptible, intermediate, resistant, and immune. Crosses between susceptible varieties produced only susceptible offspring. Intermediate varieties crossed together produced both intermediate and susceptible progeny. When resistant varieties were crossed with susceptible ones, susceptibility was dominant, with only about 2 per cent of the progeny as resistant as the resistant parent. When susceptible varieties were crossed with the immune ones—Martin, Hussar, and White Odessa—there seemed to be a dominance of resistant plants. More than half of the hybrids in the third and subsequent generations produced less than 5 per cent of bunted heads, whereas about 20 per cent were bunt free. In crosses between immune and resistant varieties an occasional segregant showed more susceptibility than the resistant parent. Gaines believed that the results indicated that immunity or resistance to bunt is due to the combined effect of several unit factors which, when

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³ Reference is made by number (italic) to "Literature cited," p. 232.

all are present as in immune varieties, produce apparent dominance, but a lesser number would give the recessive effect. On the other hand, the writer (2) found that Martin has one dominant factor for resistance, whereas Hussar has the same factor that Martin has and probably one other.

Gaines and Singleton (6) reported that "Marquis is fairly susceptible when fall sown, but does show a small amount of resistance in that three of the four replications were in the 70.5 per cent class. Marquis, when spring sown, falls into the 8 per cent class. Turkey, which falls into the 8 per cent class if fall sown, is immune in the spring sowing." An analysis of F_2 families from a cross of Marquis \times Turkey showed some segregates more susceptible and others more resistant than the parents. The factor for resistance in Turkey was thought to be about four times as "prepotent" as the Marquis factor. A correlation coefficient of 0.711 ± 0.027 between fall-sown and spring-sown rows indicated the action of the same genes, the spring conditions appearing only to intensify their expression.

METHODS AND MATERIALS

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. Conditions there favor such investigations because relatively high bunt infection can be obtained when wheat is sown in the fall. Both spring and winter varieties may be seeded at that time without any danger of winterkilling and with the assurance that both types will mature in the following summer.

The seeds were thoroughly blackened with bunt by placing an excess quantity of the spores with the wheat in a glass container and shaking it vigorously. The inoculum, *Tilletia tritici*, was collected by W. W. Mackie in 1917 on Little Club wheat in the Montezuma Hill district of Solano County, Calif. This was originally propagated by Mackie on the Little Club variety of wheat in the botany garden at Berkeley, Calif. Since 1919 the writer has propagated smut from this same collection on White Federation wheat at Davis. The inoculum used, therefore, has been derived from one original collection of bunt. At first this procedure was followed not because it was suspected that there were physiologic races of bunt, but because this collection method offered an accessible and definite source of spores. More recently, Faris (3), Rodenhiser and Stakman (8), Reed (7), and Gaines (5) have reported physiologic forms of this fungus. The fact that the same collection of bunt has been used continuously at Davis makes it reasonably certain that the same form or mixture of forms has been employed in all the writer's investigations. This is indicated also by the consistent way in which the parental wheat varieties have reacted to this inoculum.

The wheat seeds were spaced from 2 to 3 inches apart in rod rows 1 foot apart. The entire nursery was sown within three or four days in order to avoid the effects of different temperatures and soil moistures. The nursery always was sown in a field where no wheat had been grown during the previous year, so that it was almost entirely free from volunteer grain.

At harvest time the plants in each row were pulled and separated into two piles, bunt free and bunted. The total number of plants and the number of bunted plants were recorded, and the percentage

of bunt infection was calculated. A plant was classified as bunted if it showed any infection.

Hussar was one of two varieties of wheat found to remain free from bunt when inoculated with spores of *Tilletia tritici* in an extensive test conducted by the Washington, Oregon, and California agricultural experiment stations in cooperation with the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture (9). Hussar has been entirely free from bunt over a 9-year period when inoculated with the bunt collection used in these experiments. It has been attacked by some collections of bunt used elsewhere (5, 7). Little Club, the susceptible variety used in these experiments, has produced from 70 to 95 per cent of bunted plants when the same inoculum was employed that produced no bunt in Hussar.

A strain lacking the factor for resistance common to Hussar and Martin, but having the second resistance factor carried by Hussar, was isolated from a cross between Hussar and Hard Federation. Twenty resistant F_4 plants of this cross were crossed with Martin and tested for bunt resistance in the F_2 . The progenies from plants numbered 1403, 1418, and 1511 of the Hussar \times Hard Federation cross contained about 7 per cent of bunted plants, which indicated that these plants lacked the Martin factor. The other 17 progenies did not segregate, indicating that here both parents carried the Martin factor for resistance. At the time the parent F_4 plants were crossed with Martin they were crossed also with Little Club to supply material for a genetic study of resistance to bunt.

Unlike Hussar, selection 1418 is not immune from bunt, but it is highly resistant. Four rod rows produced 0.95 per cent of bunted plants in 1927, and 13 rows averaged 1.7 per cent in 1928. The fact that a little bunt appeared in this selection might suggest that this factor is not so effective in controlling bunt as is the Martin factor. Such, however, may not be the case, for these plants were from material which had been selected the previous year from rows with a slight percentage of bunt, and they were being grown to determine whether this percentage might not be due to modifying factors. The selection 1403 has been entirely bunt free. This selection would have been used in the study here reported, had not the cross of it with Little Club failed to yield seed because of an accident. The slight percentage of bunt in selection 1418 has not disturbed the results materially.

EXPERIMENTAL RESULTS

F_2 PROGENIES

All F_2 progenies were grown in 1927. The resulting data are presented in Table 1.

TABLE 1.—Total number of plants and number and percentage of bunted plants in parents and F_2 of the crosses named

Parent or cross	Number of plants		Percentage of bunted plants
	Total	Bunted	
Selection 1418.....	210	2	0.95
Little Club.....	242	202	83.50
Selection 1418 \times Little Club.....	702	406	53.30
Selection 1418 \times Martin.....	81	6	7.40

In the F_2 progeny of selection 1418×Little Club there were 53.3 per cent of bunted plants, as compared with 19.2 per cent in the F_2 of Martin×Hard Federation and 17.2 per cent in Martin×White Federation (2). No doubt some susceptible plants escaped infection. On the other hand, some resistant plants probably became infected because selection 1418 is not immune. The data therefore indicate that the factor for resistance in selection 1418 allows bunt to develop on about 50 per cent of the heterozygous plants.

In the F_2 progeny of selection 1418×Martin there were 7.4 per cent of bunted plants, as compared with 9.7 per cent in both Hussar×Hard Federation and Hussar×Baart (2). However, there were only 81 plants as compared with about 2,000 in each of the Hussar crosses. The data suggest that Martin and selection 1418 contain the two main factors for immunity and resistance present in Hussar.

SEGREGATION IN F_3

Data collected in F_2 indicate the genetic segregation only roughly, because some susceptible plants apparently escape infection. In the most susceptible varieties it is rare that 100 per cent of the plants in a row are infected. If seed of the disease-free plants of such a susceptible variety is selected and sown the following year, the resulting crop does not differ from the unselected variety in resistance to bunt. F_2 data are valuable, however, because they give some indication of the number of factors for resistance present. They also indicate the percentage of bunt to be expected in heterozygous F_3 rows.

In 1928 there were grown 300 F_3 rows of selection 1418×Little Club from seeds of 300 F_2 plants not subjected to bunt infection in F_2 , and 81 F_3 rows of selection 1418×Martin. The results are recorded in Table 2.

TABLE 2.—*Distribution of the rows of the parents and of the F_3 hybrids named into percentage classes for bunt infection*

Parent or cross	Distribution of rows by percentage classes for bunt infection														Total number of rows
	0	<5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	
selection 1418.....	6	6	1												13
Little Club.....	23	31	11	8	1	6	8	14	15	27	31	27	15	8	8
Selection 1418×Little Club.....	34	10	11	4	6	5	2	2	1	0	0	2	1	1	300
Selection 1418×Martin.....															81

The distribution of the rows of selection 1418×Little Club into percentage classes of bunt infection is shown by the solid line in Figure 1. This may be compared with the distribution of Martin×White Federation, which is shown by the broken line and which is reprinted from the earlier publication (2, fig. 2). The class distributions are given in percentages because of the difference in size of the two populations.

The curve for the cross selection 1418×Little Club is distinctly trimodal. The distribution of rows under the three modes agrees very closely with a 1:2:1 ratio. The first mode represents 74 rows where 75 were expected. The second mode represents 147

rows where 150 were expected, and the third mode represents 79 rows where 75 were expected. The deviations between the obtained and calculated numbers are very small. However, the two minimums on the curve should not be thought of as being the exact limits between the three phenotypes.

The spread of each group exceeds that which at first might be expected. The resistant parent 1418 did not show percentages of bunted plants beyond the 5 to 10 per cent class, while the resistant hybrid rows, as set off by the first minimum, include percentages up to 15 to 20 per cent. The 15 rows of F_2 plants grown in 1927 contained from 31.7 to 66 per cent of bunted plants, while the heterozygous F_3 rows ranged from 17.5 to 62.5 per cent. Finally, the rows of the susceptible parent contained from 77.5 to 92.5 per cent of bunted plants, as compared with 62.5 to 92.5 per cent for the susceptible F_3 rows. If larger numbers of parent rows had been grown, it is entirely

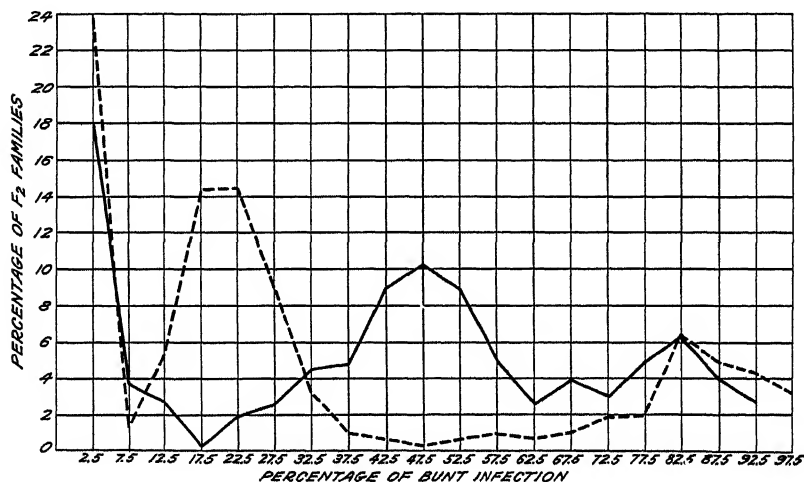


FIGURE 1.—Distribution of F_2 families of the crosses selection 1418×Little Club wheat (solid line) and Martin×White Federation wheat (broken line) into 5 per cent classes for bunt infection on the basis of the percentage of infection occurring in the F_3 rod rows

possible that the extremes would have approached those of the hybrid rows. Data to be published elsewhere show that modifying factors may be present. The presence of modifying factors would increase variability, as would the presence of field hybrids or occasional mechanical mixtures which might have occurred in threshing and seeding. Considering all variations, the data indicate that selection 1418 differs from Little Club in one main factor for resistance to bunt.

If the resistance factor of selection 1418 and the Martin factor are the only factors determining resistance to bunt in Hussar, a cross between selection 1418 and Martin should give results similar to those obtained by crossing Hussar with a susceptible wheat. Such a cross was made, and small F_2 and F_3 populations were grown. In F_2 , as shown in Table 1, 7.4 per cent of bunted plants occurred, as compared with 9.7 per cent in both Hussar×Baart and Hussar×Hard Federation (2). The distribution of F_3 rows by percentage classes for

bunt infection is given in Table 2. This distribution is similar to that obtained when Hussar was crossed with Baart (2), as may be seen from Figure 2. For comparison, this latter curve is reprinted from the earlier publication (2, fig. 4).

With two factors for reaction to bunt present, it is difficult to determine the limits of the phenotypes, because they overlap, due to the variable amount of bunt produced in a single genotype. However, the susceptible and the resistant rows may be determined fairly accurately. If the rows containing from 50 to 85 per cent of bunt are considered as homozygous susceptible, there are six strains where five were expected. Defining the susceptible group as one containing 60 to 95 per cent of bunt, to correspond with the susceptible group in

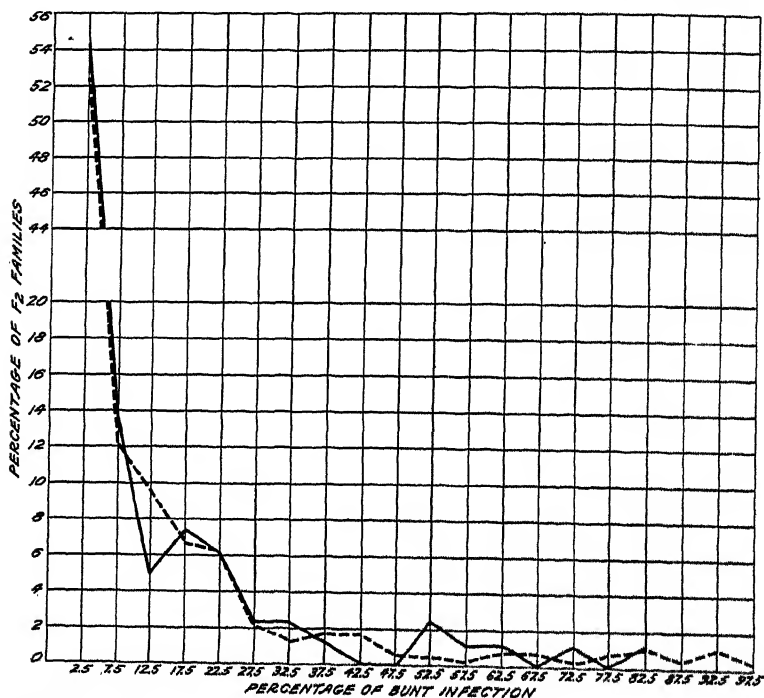


FIGURE 2.—Distribution of F₃ families of the crosses selection 1418 x Marlin wheat (solid line) and Hussar x Baart (Early Baart) wheat (broken line) into 5 per cent classes for bunt infection on the basis of the percentage of infection occurring in the F₃ rod rows

the cross 1418 x Little Club, gives three susceptible rows where five were expected. This latter is a deviation of 2 ± 1.47 , which is a satisfactory agreement. There are 34 smut-free rows where 35 were expected. Past experience indicates that the resistant group should not be made up entirely of smut-free or completely resistant rows, but should include some rows with a low percentage of bunt. Considering that only 81 rows were available as compared with 598 from the cross Hussar x Baart, the similarity between the curves is close. The data therefore show that the Martin factor and the resistance factor in selection 1418 are the two factors that cause resistance in Hussar, at least so far as the smut collection used in these experiments is concerned.

DISCUSSION

The resistance of cereals to smut may be due to multiple genetic factors in some cases. Wakabayashi (10) concluded that the resistance of Red Rustproof oats to covered smut, *Ustilago levis*, depended on three independent dominant factors. Barney (1) also found three independent dominant factors in oats for resistance to loose smut, *U. avenae*. Gaines (4) believed that resistance to bunt, in wheat varieties such as Martin, Hussar, and White Odessa is due to multiple factors.

Under the present conditions for studying the inheritance of resistance to bunt, the presence of multiple factors for resistance in a variety may make it impossible to determine with certainty the number of factors present and the exact effect of each. Because of the variability of the percentage of bunt in strains of the same genotype, there is likely to be an overlapping of phenotypes where two or more factors are present, with the result that it is impossible to establish the limits of the various phenotypes. Until methods are developed whereby 100 per cent of the susceptible plants become diseased, so that the genetic analysis may be made in F_2 , it may be necessary to isolate each factor for reaction to bunt and to study it alone.

Hussar differs from the susceptible varieties of wheat in two main factors for resistance to the particular collection of bunt used in these experiments. One of these is the same as the completely dominant factor in Martin, and the other allows the disease to develop on about 50 per cent of the heterozygous plants. This does not preclude the possibility of still other factors which might become apparent in the presence of other physiologic forms of bunt.

The factor for resistance in Martin hereafter will be designated as *MM*. The second factor in Hussar, exemplified in selection 1418, will be designated as *HH*. Martin then may be designated as *MMhh* and Hussar as *MMHH*.

Some progress has been made in the production of bunt-resistant wheats, and through continued breeding more resistant commercial varieties should become available within the next few years. As physiologic races of bunt exist, it seems very desirable to isolate and study the effect of as many different factors for resistance as possible. The more factors for resistance the plant breeder has at his disposal, the more likely is he to succeed in breeding bunt-resistant wheats in the presence of two or more physiologic forms of this disease organism.

SUMMARY

In a previous paper data on the inheritance of resistance to bunt in crosses with Martin and Hussar wheats were presented. The data available at that time showed that Hussar had the same dominant factor for resistance as the one found in Martin and probably also had one other. The exact effect of the second Hussar factor was not determined.

By appropriate breeding tests there was secured selection 1418, which contained the second Hussar factor but did not have the factor for bunt resistance common to Martin and Hussar.

Selection 1418 was crossed with susceptible Little Club, and the inheritance of resistance to bunt was studied in F_2 and F_3 . This

selection was found to differ from Little Club in one main factor for resistance to bunt. Unlike the completely dominant Martin factor, it allows about 50 per cent of the heterozygous plants to become infected.

Martin crossed with selection 1418 segregated in F_2 and F_3 similarly to Hussar \times Baart, showing that the factors in Martin and in selection 1418 are the two main factors for resistance to bunt in Hussar.

The Martin factor for resistance hereafter will be designated as *MM*, and the second Hussar factor as *IIII*. Martin then may be designated as *MMhh* and Hussar as *MMIIII*.

The isolation and study of different factors for resistance to bunt should aid in the breeding of resistant wheats where different physiologic forms of this disease organism exist.

LITERATURE CITED

- (1) BARNEY, A. F.
1924. THE INHERITANCE OF SMUT RESISTANCE IN CROSSES OF CERTAIN VARIETIES OF OATS. *Jour. Amer. Soc. Agron.* 16: 283-291, illus.
- (2) BRIGGS, F. N.
1926. INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI* (BJERK.) WINTER, IN WHEAT. *Jour. Agr. Research* 32: 973-990.
- (3) FARIS, J. A.
1924. FACTORS INFLUENCING THE INFECTION OF WHEAT BY *TILLETIA TRITICI* AND *TILLETIA LAEVIS*. *Mycologia* 16: 259-282, illus.
- (4) GAINES, E. F.
1925. THE INHERITANCE OF DISEASE RESISTANCE IN WHEAT AND OATS. *Phytopathology* 15: [341]-349.
- (5) ———
1928. NEW PHYSIOLOGIC FORMS OF *TILLETIA LAEVIS* AND *T. TRITICI*. *Phytopathology* 18: 579-588.
- (6) ——— and SINGLETON, H. P.
1926. GENETICS OF MARQUIS \times TURKEY WHEAT IN RESPECT TO BUNT RESISTANCE, WINTER HABIT, AND AWNLESSNESS. *Jour. Agr. Research* 32: 165-181.
- (7) REED, G. M.
1928. PHYSIOLOGIC RACES OF BUNT OF WHEAT. *Amer. Jour. Bot.* 15: 157-170.
- (8) RODENHISER, H. A., and STAKMAN, E. C.
1927. PHYSIOLOGIC SPECIALIZATION IN *TILLETIA LAEVIS* AND *TILLETIA TRITICI*. *Phytopathology* 17: 247-253, illus.
- (9) TISDALE, W. H., MARTIN, J. H., BRIGGS, F. N., MACKIE, W. W., WOOLMAN, H. M., STEPHENS, D. E., GAINES, E. F., and STEVENSON, F. J.
1925. RELATIVE RESISTANCE OF WHEATS TO BUNT IN THE PACIFIC COAST STATES. *U. S. Dept. Agr. Bul.* 1299, 30 p.
- (10) WAKABAYASHI, S.
1921. A STUDY OF HYBRID OATS, *AVENA STERILIS* \times *AVENA ORIENTALIS*. *Jour. Amer. Soc. Agron.* 13: 259-266.

AN AUTOMATIC WATERING SYSTEM WITH RECORDER FOR USE IN GROWING PLANTS¹

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INTRODUCTION

It is often highly desirable to use automatic balances in studies of water relations in plants. There have appeared at different times various kinds of apparatus designed to keep the soil-moisture content constant and to record the amount of moisture lost by plants. The types of apparatus described,² as a rule, have not possessed both these features, or have been inaccurate apparently due to the effects of wind or temperature.

The apparatus described in this paper was designed for use in connection with studies now being carried on in the Office of Tobacco and Plant Nutrition on the response to length of day of plants at constant temperature and humidity; in these studies close control and accurate records of soil moisture are necessary. The system is mechanically somewhat similar to the apparatus used by Blackman and Paine³ and Hamorak⁴ and is adapted both to the control of soil moisture and to the recording of moisture used; it should prove as serviceable in the field as it has in the control room. It has been in operation for about two years and even in its original form has proved reliable and required but a few minutes' attention every day or two.

The essential component of the apparatus is a swinging or rocking funnel through which a stream of water flows at a constant rate. This stream of water is diverted into a plant container for almost exactly one minute whenever the loss in weight of the container causes the beam of the balance on which it rests to close an electrical contact. The same current that operates the funnel also moves a magnetically operated pen over a moving time chart, thus producing a permanent record. The quantity of water added is then easily computed, since it is equal to the flow of water per minute multiplied by the number of times the funnel has been actuated as indicated by the chart.

APPARATUS

The system may be said to comprise the following units, each of which will be discussed in turn: (1) A minute-contact master clock,

¹ Received for publication July 1, 1929; issued February, 1930.

² BURGERSTEIN, A. DIE TRANSPIRATION DER PFLANZEN. EINIGE PHYSIOLOGISCHE MONOGRAPHIE. 3 t., illus. Jena, 1904-25.

BRIGGS, L. J., and SHANTZ, H. L. AN AUTOMATIC TRANSPIRATION SCALE OF LARGE CAPACITY FOR USE WITH FREELY EXPOSED PLANTS. Jour. Agr. Research 5: 117-132, illus. 1915.

LIVINGSTON, B. E., and HAWKINS, L. A. THE WATER-RELATION BETWEEN PLANT AND SOIL. 48 p., illus. Washington, D. C. 1915. (Carnegie Inst. Wash. Pub. 204.)

³ BLACKMAN, V. H., and PAINE, S. G. A RECORDING TRANSPIROMETER. Ann. Bot. [London] 28: [109]-113, illus. 1914.

⁴ HAMORAK, N. EIN NEUER TRANSPIROGRAPH. Ber. Deut. Bot. Gesell. 46: 2-7, illus. 1928.

(2) a 30-volt storage battery with combined charging and timing panel, (3) a rocking funnel, (4) a balance, (5) a recorder, and (6) a soil-moisture distributor.

The minute-contact master clock employed has an accuracy, under normal room conditions, of ± 20 seconds per month and closes an electrical contact, each minute on the minute, for about two seconds. However, as a clock error of even 14.4 minutes per month would correspond to an error of only 1 per cent in the quantity of water added, almost any fairly accurate clock having a minute contact can be used. If necessary, a clock having three hands can be converted into a suitable timing device by causing the second hand to dip into a mercury

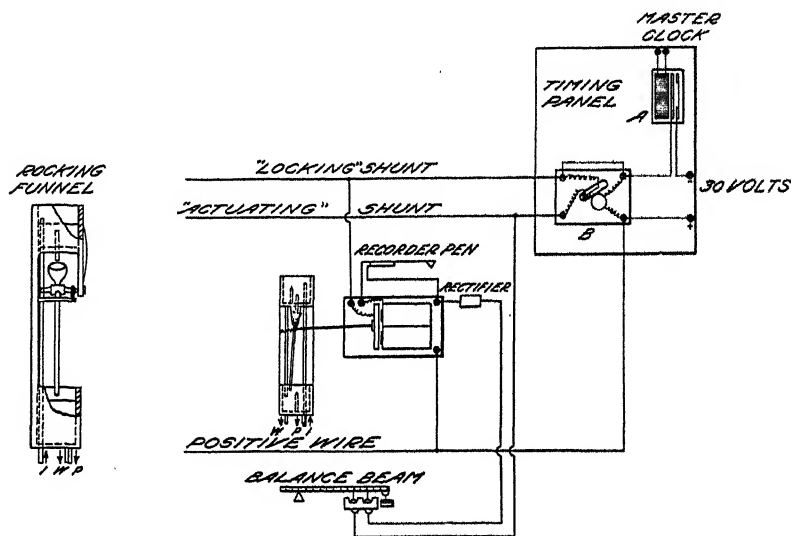


FIGURE 1.—Electrical circuits of a timing panel with one rocking funnel and balance. To the left is a side view of a rocking funnel and mounting comprising a water level with drip tube on top, support for pivoted shaft underneath, and double catch cup at the bottom. *a*, An intermittent relay operated by the minute-contact clock; *b*, mercury-vacuum relay; *i*, water inlet; *p*, outlet into plant container; *w*, waste

well once during each revolution, or by placing a cam on the second-hand shaft, which in turn opens and closes an electrical contact.

The combined charging and timing panel is shown in Figures 1 and 2. The former, with a 30-volt storage battery on trickle charge, is provided with a safety relay that opens when the line current fails. A specific description of its details is unnecessary, as they are standard and can be found in many good electrical textbooks. The use of storage batteries is considered advisable only in order to prevent interruptions due to line failure.

The timing panel consists of relays *A* and *B*, the former controlling both the "actuating" and the "locking" shunt wires to the "funnel" relays, and the latter the actuating shunt wire only. Relay *A* is an intermittent relay operated by the minute-contact clock. It alternately opens and closes each minute on the minute; that is, its contact is open for one minute, then shut one minute, then open one minute, and so on. Relay *B* is a mercury-vacuum switch having a somewhat greater time lag than relay *A*. It is operated by relay *A*,

the closing of relay *A* resulting in the opening of relay *B*. The circuit through the actuating shunt is, therefore, closed only each alternate minute for a length of time corresponding to the difference in lag of the relays, since the actuating shunt circuit must be completed through the contacts of both relays.

It will be noticed that the positive wire from the battery goes to the lower coil post of each telegraph relay rocking the funnels. The current passing through the coils of these funnel relays can flow back to the battery through either the actuating or the locking shunt. Since the beam contact is in the actuating-shunt line, the closing of the funnel relays can occur only during the instant at the beginning of each alternate minute when relay *A* contact is closing and relay *B*

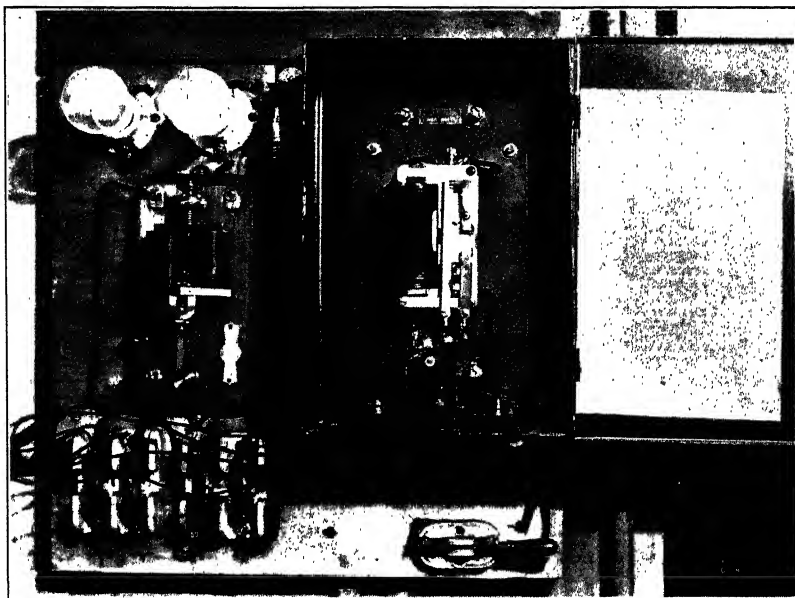


FIGURE 2.—Combined battery trickle charger with safety relay and timing relays

contact has not yet opened. Once the funnel relay is closed, however, the current passes through the funnel-relay contact and the locking shunt and locks or keeps the funnel relay closed until relay *A* contact opens at the end of the minute.

In order to prevent the feeding back of current to other watering units on these shunts, a kuprox or other dry rectifier unit, such as is used in battery charging on alternating current, is placed in series with each balance-beam contact. The use of spring contacts opened by the funnels, the method originally employed, was abandoned in favor of these units, which require less attention; that is to say, there is one contact less per automatic balance to require attention.

The rocking-funnel mounting (figs. 1 and 3) consists of a single brass casting, the top of which serves as a constant water level (1 inch deep) from which water flows at a fixed rate through a vertical vent or drip tube into the funnel suspended underneath. The inlet to the drip tube should be at least a quarter of an inch above the

bottom of the constant water level. The funnel passes through a hole in a pivoted shaft and is biased by means of a spring. With the rocking funnel in the position shown in Figures 1 and 2, the water flows into the left-hand compartment of the catch cup and the waste runs out through tube *w*. In the event, however, that the container with its soil and plants loses weight, the beam contact of the balance on which it rests is closed, thus permitting the funnel relay to be closed during the instant the actuating-shunt circuit is completed through relay *a* and relay *b* contacts. The funnel relay at the same time closes its contact to the locking-shunt line and is thus prevented from reopening until relay *a* opens at the end of the minute. Closure of the funnel relay causes the funnel outlet to swing to the right and

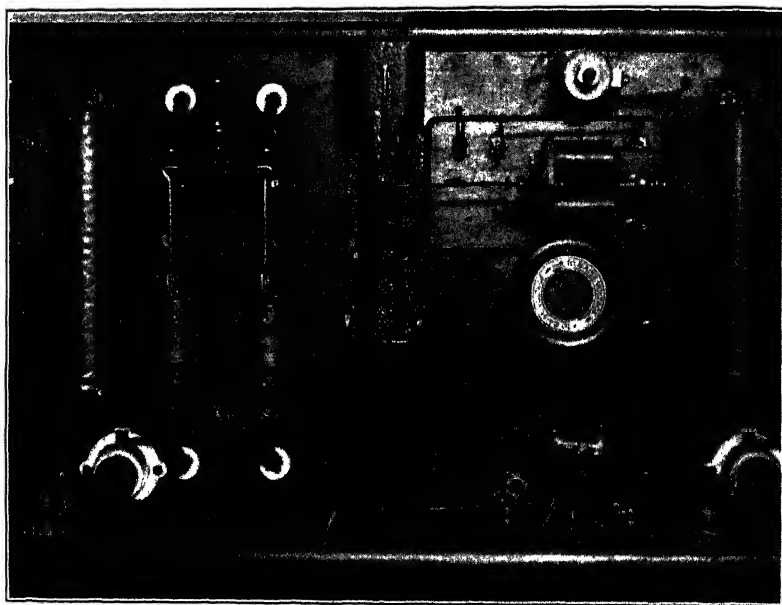


FIGURE 3.—Rocking funnel and relay as they appear on switchboard above light case. The rectifier unit, here functioning as a check valve, is just above the relay

empty into the right-hand compartment of the catch cup, whence the water flows through tube *p* into the plant container. Tube *i* is the water inlet to the water level at the top.

The balance contact consists of a nichrome wire U whose bend is twisted about another wire passing around the beam and firmly fastened to it. It is insulated from the beam, however. This U, or double contact, dips into two iron cups filled with mercury and supported in a fiber block that is bolted to the frame of the balance.

As designed, this system of automatic watering can readily be used with a recorder. The recorder should preferably be what is known as a time recorder⁵ and is used only to record the times at which the funnels are rocked. Since, however, the water presumably is

⁵ Since this paper was written, small, inexpensive ratchet counters have been found satisfactory when readings at regular intervals are practicable. These counters are actuated mechanically by the swing of the rocking funnel.

flowing at a constant rate and flows into the plant container for exactly one minute each time the funnel is rocked, the quantity of water added is equivalent to the number of times the funnel has been rocked multiplied by the water flow per minute.

Suitable recorders are being produced commercially or can be assembled with a moving chart and magnet pens. A recorder having pen coils of 30 ohms for use on 3 volts should prove satisfactory for use in series with 250-ohm funnel relays on 30-volt battery. Three typical 24-hour records at varying rates of water flow are shown in Figure 4.

In the event that a recorder is not required, the system can be greatly simplified, since in this case only the rocking funnels with their relays and the balances will be necessary.

METHOD USED TO DISTRIBUTE THE WATER THROUGH SOIL IN THE PLANT CONTAINER

If 80 pounds of wet soil per container are used, the theoretical degree of soil-moisture control with a balance sensitive to 1 ounce is ± 0.04 per cent soil moisture on the wet basis. Ignoring the effect of increasing mass of the plant, this means that about 1.7 cubic inches of water, or 1 ounce, must be distributed through a volume of about 2,000 cubic inches; or, if only horizontal uniformity is considered, the 1.7 cubic inches of water must be spread over a soil area of 231 square inches (container about 16 by 16 inches at base) to a thickness of 0.0008 inch. Even if feasible, an absolutely uniform distribution of water throughout the soil mass would probably require costly and complicated apparatus. Therefore, like previous investigators, the writer decided to attempt only horizontal uniformity. This is, after all, the natural distribution of moisture in the soil of the field, although the objection might be raised that the roots of the plants will tend to grow into the soil level having the most favorable moisture content instead of scattering uniformly through the soil; that is, the roots are not entirely free from any influence caused by variation in soil moisture. Within certain limits the plants are not growing in soil of average moisture content represented by the whole vertical soil column.

Uniform horizontal distribution of the soil moisture was accomplished by adding the water through a copper tube of $\frac{1}{4}$ -inch outside diameter perforated at 2-inch intervals. The tube was bent into an 8-inch square with two vertical risers used as inlet tubes. These risers, which were not perforated, projected above the soil. The tubes were cleated to the bottom of the plant container, covered with a half inch of sand saturated with water, and the soil added to within about 1 inch of the top. Moisture determinations made at different times in vertical soil columns were within ± 1 per cent throughout the containers.

Evaporation from the plant containers is minimized as follows: The plant containers used in the present case are $\frac{3}{4}$ -inch cypress (outside 17 by 17 inches by 10 $\frac{1}{2}$ inches high), covered on the inside with asphalt applied hot and with two coats of white enamel on the outside. The lids, which are of galvanized iron, are coated on the inside with asphalt applied hot and on the outside with two coats of white enamel. There are altogether fifteen 2-inch holes and two $\frac{1}{8}$ -inch holes in each lid. The latter are made air-tight

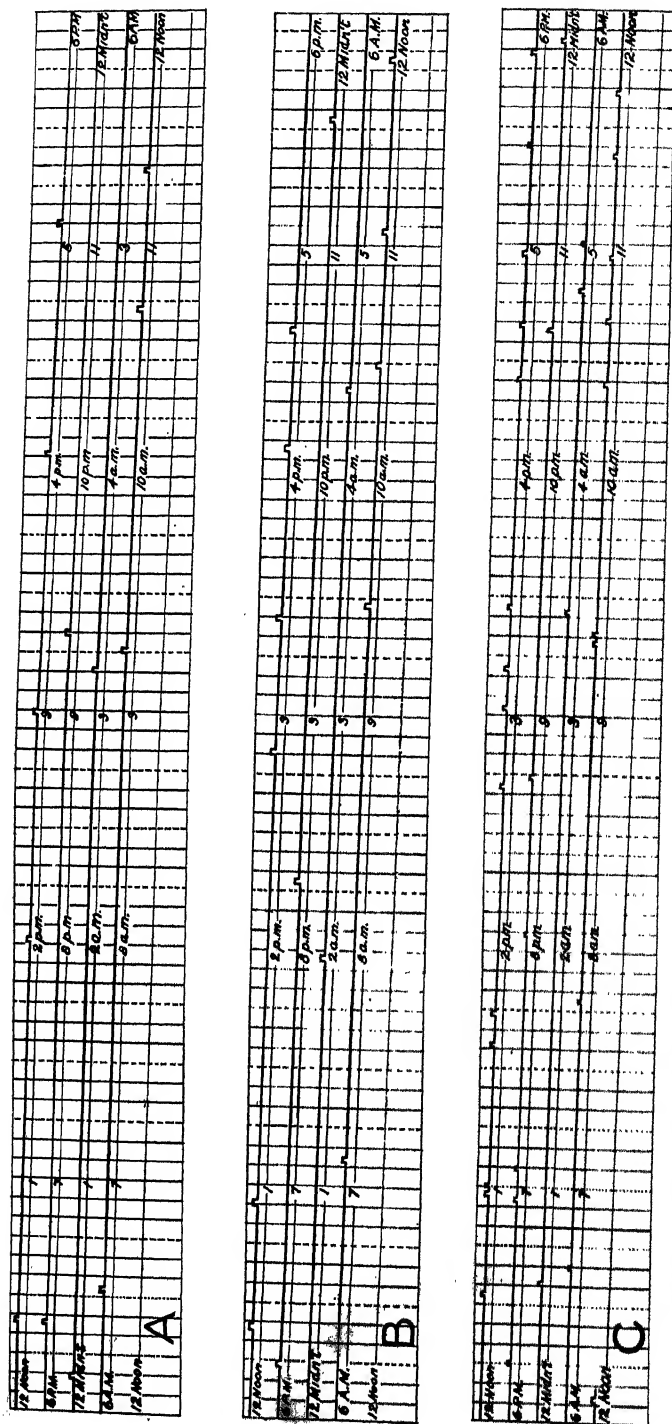


FIGURE 4.—Twenty-four-hour charts with transpiration records. Each tooth or notch corresponds to 50 gm. of water in A, 30 gm. in B, and 20 gm. in C

with rubber tubing around the risers from the watering tubes. The former are provided with perforated split paraffined corks. The corks are placed around the stems of the plants and can be packed with cotton to prevent loss of moisture. Evaporation from a container covered with a lid and unparaffined solid corks was 6,050 gm. when kept in a greenhouse from March 2, 1927, to January 11, 1928 (315 days), or less than 1 ounce a day.

Evaporation from a container at $77.0^{\circ} \pm 0.5^{\circ}$ F. and 54.2 ± 0.75 per cent relative humidity under continuous artificial illumination of about 4,500 foot-candles with a Mazda lamp screened by 3 inches of water and with an air velocity of about 90 feet per minute amounted to 126 gm. per day or 5.26 gm. per light hour. A similar determination in the dark gave 3.98 gm. per unlighted hour. The holes in the split corks were left open in both cases.

DISCUSSION

The expectation of accuracy in results depends largely upon balance sensitivity for average soil moisture and upon the rate of water flow for transpiration or evaporation requirements. As has been noted, the balance sensitivity with the weights of soil used would theoretically provide for maintaining average soil moisture constant within a variation of ± 0.04 per cent with water flow of 1 ounce per minute. This is easily checked by observation of the balance beam. Its position should at all times indicate an exact tare of the plant container, so that the removal of an ounce of material from the container will throw in the balance contact.

Accuracy of transpiration records is largely dependent upon the accuracy with which each record on the chart represents a definite amount of water. The physical factors governing flow in small tubes and nozzles are known,⁶ as is also the fact that deposition of sediment gradually reduces the rate of flow. Head is maintained constant by use of a water level with a large capacity overflow, and temperature by thermostatic control of the water temperature to $\pm 1.5^{\circ}$ C. While sedimentation could be minimized or eliminated by the use of distilled water or of one of the well-known methods of water softening, these methods are costly and require considerable attention. It therefore seemed more practicable to determine, if possible, the conditions under which clogging is least and to clean the drip nozzles as necessary.

When the tap water in Washington, D. C., is used, clogging is believed to be due to the deposition upon the walls of the drip tube of colloidal material from the previously clear water. At least it was found that a strainer having holes one-fourth the bore of the drip tube would not retain the sediment. Briefly summarized, it appeared that the apparent self-cleaning action of the drip tube was greatest when the type of flow was intermediate between drops and streamline or even flow. Constancy between duplicate tests of the rate of flow is satisfactory, and although not so good as with streamline flow it is much better than when the flow is in drops. Maximum variations between duplicates at the rates studied in grams per minute were: (1)

⁶ MARKS, L. S. MECHANICAL ENGINEERS HANDBOOK. PREPARED BY A STAFF OF SPECIALISTS. Ed. 2, p. 281. New York and London. 1924.

16.15 \pm 0.81 per cent for drops; (2) 20.20 \pm 0.50 per cent for flow intermediate between drops and streamline flow; (3) 29.91 \pm 0.57 per cent for flow as in 2; and (4) 49.80 \pm 0.14 per cent for streamline flow. Decrease in rate of flow with time was found to be, respectively, as follows: (1) Inconsistent; (2) 0.93 per cent in 7 days (0.99 per cent variation between maximum and minimum during this interval); (3) 0.38 per cent in 15 days (1.09 per cent variation between maximum and minimum during this time); and (4) 1.08 per cent in 2 days. It is probable that it is entirely practicable to obtain a water flow and therefore transpiration records accurate to \pm 0.5 per cent or better by an inexpensive method requiring very little attention. It is necessary to control the temperature of the water and to adjust for head of water and length, bore, and taper of the drip nozzle to obtain the proper type of flow. The drip nozzles should be cleaned when necessary by means of a soft copper wire slightly smaller than the bore of the drip.

The data below are given to permit duplication, and to give an idea of the relations between the dimensions of the drip nozzle and the rate of water flow. The drip tubes were made of $\frac{3}{8}$ -inch brass rod threaded for 1 inch and slotted at the top to permit adjustment for head of water. They were $1\frac{1}{4}$ inches long with untapered nozzles. The inside diameters of the tubes, in the same order as the flows given in the preceding paragraph, were 0.042, 0.0465, 0.052, and one-sixteenth inch at heads measured from top of tube of thirteen-sixteenths, seven-eighths, one-half, and seven-sixteenths inch.

Time accuracy in addition of water and in its recording is dependent upon the elimination of beam vibration or oscillation and upon variations in sensitivity of the balance.

There is nothing to prevent the outdoor use of this system of automatic balancing for soil-moisture control and record. The most important variables thus introduced are wind and rain. The first necessitates the use of an oil dashpot mounted on the balance beam, as used by Briggs and Shantz,⁷ to prevent oscillation, and the latter a shelter. This system has, indeed, two distinct advantages over former systems in that the actuating contact is very short, occurs at intervals of two minutes, and minimizes though it does not eliminate the effect of beam oscillation. If necessary, all the control apparatus can be centralized in a shelter and the balances scattered in a surrounding field. Also, with the type of balance described herein, that is, beam below pan, the protection of the balance against rain is very simply accomplished by inverting a shallow square tray on the platform of the scale with the lip of the tray just short enough to avoid touching the ground. A still more effective method would be to place the scale on a platform a few inches high and extend the lip of the pan to below the bottom of the scale.

The system herein described, while subject to refinements, has given very satisfactory results, even in its original form, for over two years, eight units being in operation simultaneously for periods of six weeks or more at a time. It requires a minimum of attention every day or two to insure accurate control of average soil moisture to \pm 0.2 per cent and transpiration records accurate to \pm 1 per cent

⁷ BRIGGS, L. J., and SHANTZ, H. L. *Op. cit.*, fig. 13.

or better. The balance beams should be looked at every few days to make sure that the containers are tared; the water temperature should be kept constant to 1° or 2° F., and a wire should be passed through the drip outlets emptying into the funnels at least once every second day. If records of less accuracy are sufficient, the temperature control can be less accurate, while the drip outlets may be inspected only two or three times a week, depending on the accuracy desired.

SUMMARY

A fully automatic watering system that can be used with a recorder has been described for use in studies of plant transpiration and growth when soil moisture must be kept constant and a record must be made of the water used. Average soil moisture can be controlled as accurately as necessary by the use of a balance of sufficient sensitivity and horizontal uniformity of soil moisture to ± 1 per cent, while transpiration records accurate to ± 1 per cent can easily be obtained. The system requires, barring accidents, not over five minutes' attention per unit every second day. It is suitable for use indoors or outdoors. It is flexible enough to be used in single or multiple units, with or without a recorder, and its component parts are comparatively inexpensive and easily available.

THE INFLUENCE OF THE POTASH CONCENTRATION IN THE CULTURE MEDIUM ON THE PRODUCTION OF CARBOHYDRATES IN PLANTS¹

By GEORGE JANSSEN, *Assistant Agronomist*, and R. P. BARTHOLOMEW, *Assistant Agronomist, Arkansas Agricultural Experiment Station*

INTRODUCTION

The chief rôle assigned to potassium in plant nutrition is its function in the synthesis of sugars, starch, and possibly other carbohydrate compounds (11, 21, 28, 29, 31).² Its relation to the synthesis of proteins has also been discussed (10, 21). That it is to a large extent involved in photosynthesis is shown by its presence in the green portions of leaves and stems (4, 23, 29). Stoklasa (29) has suggested a means by which the potassium ion may enter into the reaction of the photosynthetic process during the synthesis of sugars. He has also shown by chemical analyses that plants grown on soils low in potassium are low in total sugars and starch, while those grown on soils high in potassium are high in these compounds. Other workers report similar results (11, 28, 33). Dowding (4) has shown that all meristematic tissue of the spruce is particularly rich in potassium. Reed (26) found that potassium was necessary for germination and growth of certain mosses and for starch production in all the green plants which he studied. He further found that mitotic cell division never took place without a suitable supply of potassium.

OBJECT OF THE EXPERIMENT

Since it is a well-recognized fact that potassium performs a very important function in photosynthesis and in the formation of carbohydrates, either aiding in their synthesis or translocation, or both, it appeared important to learn what effect the gradual increase of potassium in the nutrient medium would have upon plant growth and upon the synthesis of carbohydrates. In a previous paper (2) it was pointed out that the percentage of potassium in plants increased with the amount of available potassium supplied. It was the object of the present investigation to determine to what extent increments of potassium in the plant, brought about by varying the amounts of available potassium in the nutrient medium, affect (1) the total dry weight per plant; (2) the percentage of dry matter; (3) the total percentage of sugars, starch, and hemicellulose; and (4) the total sugars and starch per plant or unit of plants.

PLAN OF THE EXPERIMENT

Plants which have been designated as high and low potassium feeders were used in these experiments; they were oats, cowpeas, soybeans, sweetclover, cotton, Sudan grass, and corn. These plants

¹ Received for publication Apr. 23, 1929; issued February, 1930. Approved by the director of the Arkansas Agricultural Experiment Station. Research Paper No. 167, Journal series, University of Arkansas.

² Reference is made by number (italic) to "Literature cited," p. 259.

were grown in water cultures, sand cultures, and field soil, to which increments of potassium were added. In some cases all three methods of culture were used for the same crop plant.

METHODS

In the nutrient-solution culture work two types of solutions were used. Freedom from the sodium ion and the ability of the plant to make good growth in the medium were the basis upon which the solution was selected. It is believed that the presence of sodium ions (12) in a nutrient to which potassium is added for the purpose of study may lead to erroneous conclusions. A discussion of this possibility was presented in another paper (2).

The nutrient solution used for the first series of soybeans and oats grown in sand (Tables 1, A, and B, and 5, A, and B) contained the following compounds: Solution 1: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 147 gm.; $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, 26.9 gm.; dissolve and make to 2,280 c. c. Solution 2: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 90 gm.; dissolve and make to 2,280 c. c.

Fifty cubic centimeters each of solutions 1 and 2 were added to 1 liter of water, giving a salt concentration of 0.58 per cent. The potassium was added from a stock solution of potassium chloride. The stock solution contained 1.91 gm. KCl per liter, equivalent to 1,000 mgm. potassium per liter, or 1,000 parts per million. Thus 1 c. c. of this solution diluted to 1,000 c. c. gave a concentration of 1 mgm. of potassium per liter, or 1 p. p. m.

The jars to which the nutrient solutions were added were provided with an outlet at the bottom (modified after the method of McCall (22), to which suction could be applied, thus facilitating the removal of the old solution before more was added. The jars were brought to a specific weight before and after the renewal of the nutrient solution; hence the moisture content was uniformly maintained.

The oats were planted on December 20, 1926, and on January 28, 1927, when the more vigorous plants could be differentiated from the weaker ones, they were thinned to 18 plants per jar. On February 9 the first plants were harvested, nine being taken from each jar; on March 10 a second harvest was made; and on April 12 the remaining plants were taken up. Potassium and carbohydrate analyses were made on plants collected on all three dates. The results are presented in Table 1, A. The potassium was applied as follows: To jars 51 to 53, none; 56 to 58, 1 p. p. m.; 59, 61, and 62, 3 p. p. m.; 63, 65, and 66, 5 p. p. m.; and 68 to 70, 10 p. p. m.

The soybeans in sand cultures were treated in exactly the same manner as the oats. The seed was inoculated and planted on February 2, 1927, and after emergence the plants were thinned to six per jar. The first plant samples were taken on March 22 and the second on April 12, 1927. The potassium was applied as follows: To jars 131 and 132, none; 135 and 136, 1 p. p. m.; 139 and 140, 3 p. p. m.; 143 and 144, 5 p. p. m.; and 147 and 148, 10 p. p. m. These data are given in Table 5, A.

A series of soybeans (jars 149 to 154, Table 5, B) and a series of oats (jars 71 to 76, Table 1, B) were grown in sand through which the nutrient solution was allowed to percolate continuously. This was arranged by means of siphons, and the flow was controlled by varying the size of the channel outlet of rubber tubing by means of a Hoffman clamp.

TABLE 1.—Variations in the carbohydrate constituents, dry matter, and potassium content of oat plants grown in sand cultures moistened with a balanced nutrient solution containing different amounts of potassium

A. GROWN IN SAND CULTURE WITH NUTRIENT SOLUTION APPLIED DAILY

Jar No.	Date harvested	Potas- sium in solution	Plants per jar	Dry mat- ter	Dry weight per 10 plants	Total sugars	Dex- trins	Starch	Dex- trins and starch	Sugars, dex- trins, and starch	Hemi- cellu- lose	Potas- sium	Sugars, dex- trins, and starch per plant
		<i>P. p. m.</i>	<i>No.</i>	<i>P. ct.</i>	<i>Gm.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>Mgm.</i>
53	Feb. 9	0	45	—	0.48	8.72	—	—	3.38	12.10	8.54	—	5.86
56	do	1	45	11.1	.57	10.33	—	—	1.48	11.81	9.21	0.64	6.83
61	do	3	45	11.1	.75	8.21	—	—	2.89	11.10	10.12	.83	8.32
63	do	5	45	10.8	.90	6.14	—	—	2.04	8.18	8.49	1.38	7.36
68	do	10	45	10.9	1.07	5.41	—	—	2.87	8.28	8.25	1.73	8.85
52	Mar. 10	0	9	27.0	1.4	8.00	—	—	2.12	10.12	9.70	—	14.16
57	do	1	9	21.6	3.5	10.04	—	—	1.40	11.44	10.87	.43	41.32
62	do	3	9	20.3	8.4	10.18	—	—	1.60	11.78	10.80	.60	98.95
66	do	5	9	19.1	10.7	11.52	—	—	2.15	13.67	10.05	.71	146.26
69	do	10	9	17.2	12.4	10.01	—	—	2.87	12.87	10.13	1.12	159.58
51	Apr. 12	0	9	—	1.7	—	—	—	—	—	—	—	—
58	do	1	9	—	—	—	—	—	—	—	—	.49	—
59	do	3	9	24.1	16.8	4.18	—	—	2.07	6.25	13.15	.39	105.00
65	do	5	9	22.0	27.5	3.89	—	—	2.86	6.75	14.72	.63	185.62
70	do	10	9	22.5	45.8	4.49	—	—	4.67	9.16	15.34	.85	419.52

B. GROWN IN SAND CULTURE WITH CONTINUOUS PERCOLATION OF NUTRIENT SOLUTION

71	Feb. 9	1	18	—	1.06	10.13	0	3.86	3.86	13.99	8.56	0.63	14.82
74	do	3	18	—	1.40	13.63	.43	4.97	5.40	18.03	9.18	.93	28.50
76	do	5	18	—	1.60	8.75	.16	1.89	2.05	10.80	9.17	1.10	17.28
72	Feb. 10	1	18	—	1.53	9.76	1.30	1.09	2.39	12.15	7.83	.63	18.88
73	do	3	18	—	2.00	13.14	.37	1.12	1.49	14.63	7.81	.99	29.30
75	do	5	18	—	1.76	8.65	.17	1.36	1.53	10.18	7.32	1.10	17.91

To maintain a uniform potassium concentration in the nutrient solution it was necessary to use water cultures. Soybeans and oats did not grow well in water cultures containing the above culture solution; therefore a modification of a solution devised by Hoagland and Martin (14) and Parker (25) was utilized. This solution was as follows: Solution A: 807 gm. Ca (NO₃)₂·4H₂O; 143 gm. Mg (NO₃)₂·6H₂O; 5 gm. CaCl₂; 4 gm. MgCl₂; 1 gm. H₃BO₃ (boric acid); water to make 2 liters. Solution B: 300 gm. MgSO₄·7H₂O; 1.2 gm. MnSO₄; water to make 2 liters. Solution C: 7 gm. CaH₄(PO₄)₂·H₂O; water to make 2 liters.

It was necessary to keep these solutions in separate containers in order to prevent the precipitation of some of the salts. Ten cubic centimeters of each of the above solutions were added to 7.5 liters of distilled water, the approximate amount necessary to fill one 2-gallon jar. The potassium was added as needed from either one of two stock solutions of potassium chloride. One solution contained 28.62 gm. of pure KCl per 2 liters, and 1 c. c. diluted to 7.5 liters was equivalent to 1 mgm. potassium per liter or 1.0 p. p. m.; the other contained 2.862 gm. KCl per 2 liters, and 1 c. c. when diluted to 7.5 liters was equivalent to 0.1 mgm. potassium per liter, or 0.1 p. p. m.

To maintain the amount of potassium in the solution as nearly constant as possible, it was renewed twice daily. To accomplish this, the following procedure was adopted: Two jars were filled with 7.5 liters of distilled water, and the required amount of the nutrient

solution, excluding potassium was added. The plants from the old potassium-free jar were then placed in the new potassium-free jar just renewed. This could easily be accomplished, because the plants were growing through holes in the wooden jar covers. (Fig. 3.) The old potassium-free jar was then made to 0.5 p. p. m. by the addition of the necessary amount of stock KCl solution. The plants were then transferred to this new 0.5 p. p. m. of potassium solution. To the old 0.5 p. p. m. jar an additional 0.5 p. p. m. of potassium was added, and it then became the 1 p. p. m. jar. Similarly, to the old 1 p. p. m. solution an additional 1 p. p. m. of potassium was added, and it became the 2 p. p. m. solution. This procedure was used for all jars. The solution in each 5.0 p. p. m. jar was discarded twice daily.

In order to keep the iron in solution, the jar containing the potassium-free solution was made to pH 4 every third day by the addition of normal HCl. Fifty cubic centimeters of a 0.5 per cent solution of ferric tartrate was then added. The plants were rotated so as to keep them feeding on the specified parts per million of potassium, and in the process of rotation all passed through the jars containing the iron every third day. No plant remained in the solution of pH 4 longer than 12 hours.

The plants grown in the water-culture solution were soybeans, cowpeas, cotton, and Sudan grass. These data are given in Tables 3, A and C, 4, A, and 5, C.

TABLE 2.—Variations in the carbohydrate constituents, dry matter, and potassium content of oat and sweetclover plants when grown on Clarksville silt loam fertilized with different amounts of potassium

A. OATS GROWN ON FIELD SOIL

Fertilizer	Sampling date	Plants	Dry matter		Dry weight per 10 plants		Reducing sugars		Total sugars		Dextrins		Starch	Dextrins and starch	Hemicellulose	Potassium		Sugars, dextrins, and starch	Sugars, dextrins, and starch per plant
			No.	P. ct.	Gm.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.				P. ct.	P. ct.		
Check.....	1927 June 30	50	37.30	22.69	0.152	0.220	1.287	0.963	2.250	15.95	2.69	2.470	56.01						
NP.....	do	50	36.45	11.86	.152	.185	1.518	.750	2.268	17.15	2.79	2.453	29.09						
NP K 150.....	do	50	31.30	17.53	.083	.152	.913	.663	1.576	18.60	3.93	1.728	30.29						
NP K 300.....	do	50	28.62	19.45	.152	.152	1.000	.825	1.825	16.25	1.16	1.977	38.45						
NP K 450.....	do	50	31.30	22.09	.152	.220	1.181	.775	1.956	15.05	2.98	2.176	48.05						

B. OATS GROWN ON FIELD SOIL TAKEN TO THE GREENHOUSE

Check.....	8	3.39	7.75	3.70	8.97	2.27	11.45	38.64
NP.....	8	8.65	7.38	3.66	8.70	4.05	11.04	95.49
NP K 100.....	8	9.17	5.26	5.74	8.73	5.20	11.00	100.87
NP K 150.....	8	9.00	5.42	2.05	10.57	5.55	7.48	67.32

C. HUBAM SWEETCLOVER GROWN ON FIELD SOIL

			1927																
Check	July 8	40	23.22	13.20	3.86	4.36					7.15	12.38	2.12	11.51	152.0				
NP	do	40	24.30	13.00	3.26	4.70					5.77	12.08	2.12	10.47	136.4				
NP 150	do	40	29.00	18.50	3.04	4.95					5.49	12.26	1.92	10.44	193.1				
NP 300	do	40	24.42	22.00	2.59	4.15					6.46	11.88	1.61	10.61	233.5				
NP 450	do	40	21.05	16.50	3.82	5.30					5.33	12.00	1.70	10.63	175.5				
			1928																
Check	July 31	100	29.0	15.90	1.187	2.475	1.794	1.406	3.200	13.620	1.75	5.675	90.23						
NP	do	100	31.5	11.15	1.900	3.437	1.738	1.487	3.225	12.087	1.02	6.662	74.28						
NP 150	do	100	31.5	13.86	2.187	3.580	2.000	1.963	3.963	10.275	1.57	7.543	104.54						
NP 300	do	100	28.5	10.37	1.500	2.850	1.953	1.906	3.869	10.562	1.57	6.719	69.70						
NP 450	do	100	30.0	15.00	1.625	3.250	1.888	1.666	3.554	13.470	2.30	6.804	102.06						

TABLE 3.—Variations in carbohydrate constituents, dry matter, and potassium content of cowpeas and cotton plants grown on field soil and in nutrient solutions containing different amounts of potassium

A. COWPEAS GROWN IN WATER CULTURE

Potassium in solution and fertilizer	Sampling date	Plants	Dry matter	Dry weight per 10 plants	Reducing sugars	Total sugars	Dextrins	Starch	Dextrins and starch	Sugars, dextrins, and starch	Hemicellulose	Potassium	Sugars, dextrins, and starch per plant
P. p. m.:		No.	P. ct.	Gm.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	Mgm.
0.....		8	14.4	6.92	5.103	6.943	1.280	4.629	5.909	12.852	8.656	0.20	88.9
0.5.....		8	12.9	24.90	7.788	11.004	1.277	2.700	3.977	14.981	9.033	.71	373.0
1.....		8	12.6	38.20	9.147	12.566	1.051	2.239	3.290	15.856	7.992	.81	605.4
2.....		8	11.1	44.00	9.267	12.450	1.165	2.449	3.614	16.064	7.484	1.08	706.8
3.....		8	11.8	41.00	8.522	11.853	1.116	2.669	3.785	15.638	6.504	1.20	640.8
5.....		8	12.3	54.00	8.017	10.354	1.266	4.541	5.807	16.161	7.989	1.34	872.6

B. COWPEAS GROWN ON FIELD SOIL

	1928												
Check.....	Aug. 16	59	15.25	94.6	1.087	2.975	1.831	3.150	4.981	7.956	7.412	2.25	754.00
NP.....	do	59	14.75	96.6	1.047	3.775	1.888	3.031	4.919	8.694	7.370	2.29	839.84
NP K 150.....	do	59	14.00	87.6	1.047	3.700	2.112	3.218	5.330	9.630	5.150	2.95	791.62
NP K 300.....	do	59	14.25	87.6	.995	3.850	1.950	3.162	5.112	7.962	7.000	3.00	697.47
NP K 450.....	do	59	13.00	91.2	1.700	2.470	1.920	3.837	5.757	8.227	4.587	3.58	750.30
Check.....	Aug. 31	39	15.00	43.6	1.362	3.430	2.037	6.306	8.943	11.772	6.090	2.18	433.21
NP.....	do	39	15.00	43.6	1.362	3.430	2.037	6.306	8.943	11.772	6.090	2.18	433.21
NP K 150.....	do	39	15.00	37.0	1.875	4.000	2.331	5.625	7.956	11.956	6.550	2.32	442.37
NP K 300.....	do	39	15.00	41.0	1.225	3.075	1.218	5.462	6.680	9.755	5.420	2.32	396.95
NP K 450.....	do	39	14.50	42.5	.900	2.700	1.350	5.300	6.650	9.350	6.850	3.02	391.00

C. COTTON GROWN IN WATER CULTURE

P. p. m.:													
0.....		8	20.9	5.7	2.100	2.680	0.880	2.290	3.170	5.830	7.450	0.78	33.23
0.5.....		8	19.7	10.0	2.616	3.133	.966	2.170	3.136	6.269	9.441	.81	62.69
1.....		8	19.0	11.6	2.768	3.087	1.260	3.218	4.478	7.565	10.625	1.18	82.94
2.....		8	18.3	15.5	2.900	3.800	1.325	4.387	5.712	9.512	11.000	1.10	147.83
3.....		8	17.1	18.0	2.375	2.900	1.550	3.250	4.800	7.700	10.700	1.48	123.20
5.....		8	17.3	16.6	2.775	3.750	1.033	2.750	3.783	7.533	10.050	1.80	124.50

The crops used in field study were soybeans, cowpeas, oats, corn, sweetclover, and Sudan grass. These crops were grown in quadruplicate. In some instances samples for analysis were collected from all four plots at different stages of plant growth. The series of treatments given are: No treatment (check); nitrogen and phosphorus only; nitrogen and phosphorus with 150 pounds KCl; nitrogen and phosphorus with 300 pounds KCl; and nitrogen and phosphorus with 450 pounds KCl to the acre. The results are given in Tables 2, A, B, C; 3, B; 4, B, C; and 6, A, B.

All plant tissue obtained from the cultures-solution work used for carbohydrate analyses was preserved in alcohol which was boiling when added. The field samples were dried after the method described by Link (20). The method of carbohydrate analysis was essentially the same as that of Murneek (24) and Janssen (16).

In discussing the relation of carbohydrate production to the percentage of potassium in the plant, emphasis is placed on the total percentage of sugars, dextrins, and starch. These data are given as a separate column in the tables. It was thought that this method of comparison would give a better index of the effect of potassium on the

carbohydrate compounds in the plant than if each compound were discussed separately. Similarly, the term "total carbohydrate compounds" includes the total weight of sugars, dextrins, and starch per plant. The percentages of hemicellulose have not been included with the sum of the percentages of sugars and starch because hemicelluloses are of a more complex nature. The data for hemicellulose are listed, but will not be discussed, since they are so variable.

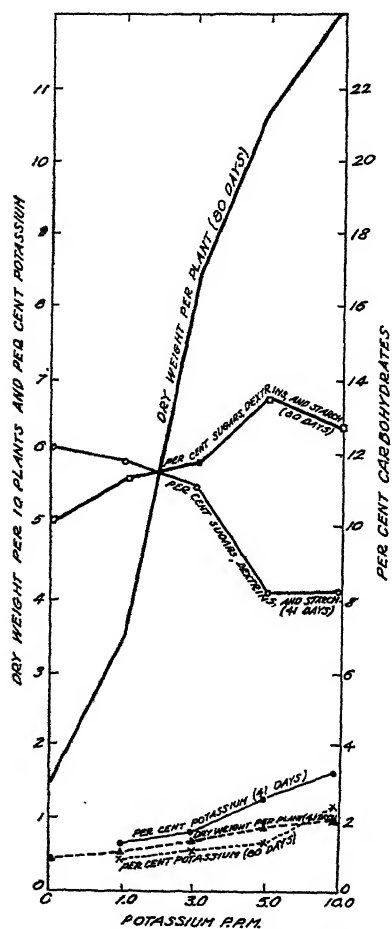


FIGURE 1.—Relation between the dry weight, potassium content, and carbohydrate constituents of oats when grown in a nutrient solution containing varying amounts of potassium

RESULTS

The data for carbohydrate and potassium analyses of plants grown in sand cultures are given in Table 1. The results of the analyses for the February 9 and March 10 collections are presented also in Figure 1, A. Large differences in growth were observed in plants receiving the various potassium treatments. (Fig. 2.) The dry weights in Table 1 show decided differences in favor of the heavier treatments of 5 and 10 p. p. m.

Table 1, B, gives data obtained from plants grown in sand but supplied with a nutrient solution which was allowed to percolate continuously on the sand in the jar. This table indicates that the maximum growth based on dry weight was obtained when the plants received the 3 p. p. m. potassium treatment. The percentage of total sugars and starch seems to bear this out.

Table 2, A and B, shows the effect of various amounts of potassium on soil cropped to oats. The soil used was classified as Clarksville silt loam. The data here shown have little significance when considered from the standpoint of the effect of potash fertilization on the growth of the plants.

Neither the sugar nor the starch shows any constant relationship to the amounts of potassium added to the soil, except that there is a slight decrease in the percentage of these compounds with the increments of potassium. It should be stated that the oat plants taken from the field were stunted by excessive summer heat and were beginning to dry up when the samples were collected.

Carbohydrate analyses were not made on sweetclover plants grown in culture solution, but the results of analyses of plants grown in field soil to which KCl was added are given in Table 2, C. Analyses were



FIGURE 2.—Fulghum oats grown on sand cultures containing the following amounts of potassium in parts per million: A, 0; B, 1; C, 3; D, 5; E, 10

made of plants collected in 1927 and 1928. The data for 1927 show the greatest dry weight per plant obtained on soil which received 300 pounds of KCl. There are no noticeable differences in the percentage of sugars and starch in plants containing different percentages of potassium. The results for 1928 show that there was little difference



FIGURE 3.—Cowpeas grown in nutrient solutions containing the following amounts of potassium in parts per million: A, 0; B, 0.5; C, 1; D, 2; E, 3; F, 5

in total plant growth resulting from potash fertilization, but that the percentage of sugars and starch increased slightly in plants grown on potash-fertilized soil.

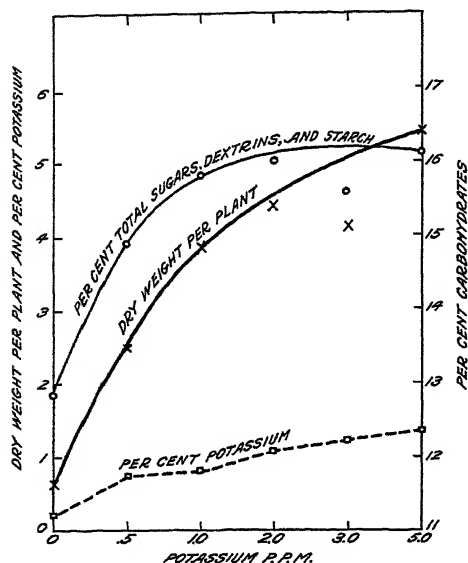


FIGURE 4.—Relations between the dry weight, potassium content, and carbohydrate constituents of cowpea plants when grown in nutrient solutions containing different amounts of potassium

The data for these plant are presented graphically in Figure 4. This figure indicates that the weight per plant, as well as the percentage of sugar and starch, increases with the increment of potassium in the plant. However, the ratio of the percentages of sugars and starch to the percentage of potassium in the plant decreased at the higher concentrations of potassium. This is evidenced by the graph showing percentage sugars and starch at the higher potassium concentrations.

Table 3, B, gives the results of the analysis of cowpea plants grown on field soil fertilized with potassium. The plants were harvested at two different stages of development, as indicated. No relation is shown between the dry weight per plant and the amounts of

The data for cowpeas grown in water-culture solutions are presented in Table 3, A. These plants made excellent growth in water cultures and were harvested 38 days after they were first placed in the solutions. Figure 3 shows the relative differences in growth of these plants. The greatest dry weight per plant was obtained from plants grown in solutions receiving 2, 3, and 5 p. p. m. of potassium. The 5 p. p. m. plants were allowed to grow 48 hours longer than the others (in order that a study might be made of the rate of potassium uptake), and for that reason the dry weight was greater than the 2 and 3 p. p. m. plants.

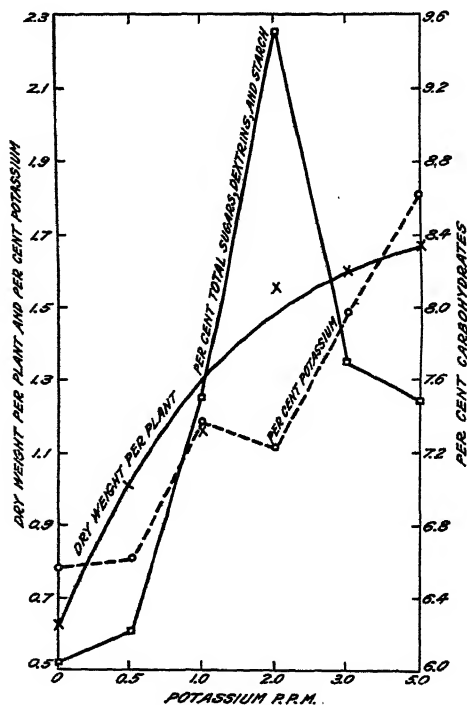


FIGURE 5.—Relations between the dry weight, potassium content, and carbohydrate constituents of cotton plants when grown in nutrient solutions containing different amounts of potassium

potassium added to the soil. Among the samples collected on August 16, 1928, the potassium-fertilized plants show a small increase in percentage of dextrans and starch, but no such increase is noted in the August 31 collection. The total sugars and starch varied in plants receiving different quantities of potassium. However, there are fairly consistent increments in the percentage of potassium in the plants with the higher rates of potassium fertilization.

Table 3, C, and Figure 5 give the data for cotton (Trice 323) grown in water cultures and harvested 81 days after planting. The dry weight per plant increased with the increments of potassium in the nutrient solution, but the sum of the percentages of sugars, dextrans, and starch decreased at 3 and 5 p. p. m. of potassium. The highest percentages of sugars, starch, and dextrans were obtained in plants grown on nutrient solutions containing 2 p. p. m. of potassium.

TABLE 4.—Percentage carbohydrate constituents, dry matter, and potassium in corn and Sudan grass grown in soil or in nutrient solutions containing different amounts of potassium

A. SUDAN GRASS GROWN WATER CULTURE

Potassium in solution or fertilizer	Part of plant	Plants	Dry matter	Dry weight per 10 plants	Reducing sugars	Total sugars	Dextrans	Starch	Dextrans and starch	Sugars, dextrans, and starch	Hemicellulose	Potassium	Sugars, dextrans, and starch per plant
P. p. m.:		No.	P. ct.	Gm.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	Mgm.
0.....	12	22.4	4.42	5.553	8.909	0.823	1.432	2.260	11.169	16.085	0.22	49.36
0.5.....	12	20.9	37.60	7.710	13.530	.658	1.987	2.645	16.175	18.910	.52	608.18
1.0.....	12	20.4	44.50	9.490	14.820	.758	1.383	2.140	16.960	16.110	.68	751.76
2.0.....	12	19.3	36.00	9.210	16.010	.680	2.249	2.929	18.939	18.320	.92	681.73
3.0.....	12	18.1	59.20	7.583	11.366	1.254	2.187	3.441	14.807	13.590	.84	876.57
5.0.....	12	18.0	60.00	6.078	11.506	1.242	1.885	3.127	14.633	17.225	1.07	877.98

B. SUDAN GRASS GROWN ON FIELD SOIL

Check.....	* 40	20.7	8.87	5.34	8.35	2.70	11.05	15.7	1.60	98.010
NP.....	40	21.3	14.60	6.10	10.58	2.97	13.55	13.2	1.36	197.830
NP K 150 ^b	40	20.3	10.50	5.25	10.49	3.58	14.07	14.1	1.95	147.735
NP K 300.....	40	19.2	9.37	6.18	9.41	2.27	11.68	13.6	2.02	109.675
NP K 450.....	40	19.2	8.01	4.00	7.94	2.90	10.84	13.4	1.98	88.820

C. CORN GROWN ON FIELD SOIL

Check.....	Stalk.....	3	15.0	497.5	13.025	17.337	1.830	1.943	3.773	21.110	13.775	2.92	*10.500
NP.....	do.....	3	15.0	339.0	11.750	16.825	2.018	1.587	3.606	20.431	13.587	2.12	6.835
NP K 150.....	do.....	3	15.0	665.0	9.712	14.287	2.825	1.037	3.562	18.149	13.325	3.70	11.887
NP K 300.....	do.....	3	15.0	775.0	9.375	12.575	2.731	1.681	4.412	17.287	13.457	4.44	13.387
NP K 450.....	do.....	3	16.0	711.4	6.250	8.862	1.737	2.131	3.868	12.730	13.475	5.03	9.056
Check.....	Leaves.....	3	22.5	409.0	1.075	2.662	1.080	1.606	2.686	5.348	14.312	2.22	2.180
NP.....	do.....	3	14.0	225.4	3.325	5.425	.700	2.469	3.169	8.594	14.612	1.78	1.937
NP K 150.....	do.....	3	18.7	387.0	2.662	3.737	.484	1.812	2.296	6.033	13.775	2.51	2.334
NP K 300.....	do.....	3	18.0	432.0	1.100	2.862	.712	2.219	2.931	5.793	13.100	2.84	2.503
NP K 450.....	do.....	3	16.0	368.0	1.700	3.662	.471	4.063	4.534	8.196	14.275	2.36	3.016

* Roots removed.

^b Pounds per acre of KCl in sections B and C.

* Unit is grams in this column of section C.

The results of the analyses of Sudan grass grown in water-culture solutions are presented in Table 4, A, and in Figure 6. These data indicate that up to a certain amount, 3 p. p. m., the dry weight per plant tends to increase with the percentage of potassium in the nutrient solution. They also show, as in the case of cotton (fig. 4), that

the percentages of sugars, dextrans, and starch is greatest in plants when grown in nutrient solutions containing 2 p. p. m. of potassium.

These results obtained on plants grown in culture solutions seem to be confirmed by those obtained from analyses of Sudan grass plants taken from field soils treated with various amounts of potassium, as shown in Table 4, B. It will be noted, first, that the total dry weight per plant bears no direct relation to the amount of potassium applied to the soil. However, the final field hay weights

show the following results: No potassium, 73.4 pounds; 150 pounds KCl, 85.8; 300 pounds KCl, 91.1; and 450 pounds KCl, 91.1. These results indicate that maximum plant development was reached when potassium was applied at the rate of 300 pounds KCl per acre. The highest percentage of sugars and starch was obtained when the plants were grown on soil which received a 150-pound application of KCl.

Corn was not grown in either water or sand cultures, but plants were collected from a field fertilized with KCl. The plant samplings were made a short time before tasseling, and the leaves and stalks were analyzed separately. The results are presented in Table 4, C. The stalks gradually decreased in total sugars and starch with increase of potassium in the plant. In the leaves there is noted a fluctuation in percentage of sugar and starch, but the percentage of these compounds in plants grown on soils receiving a complete

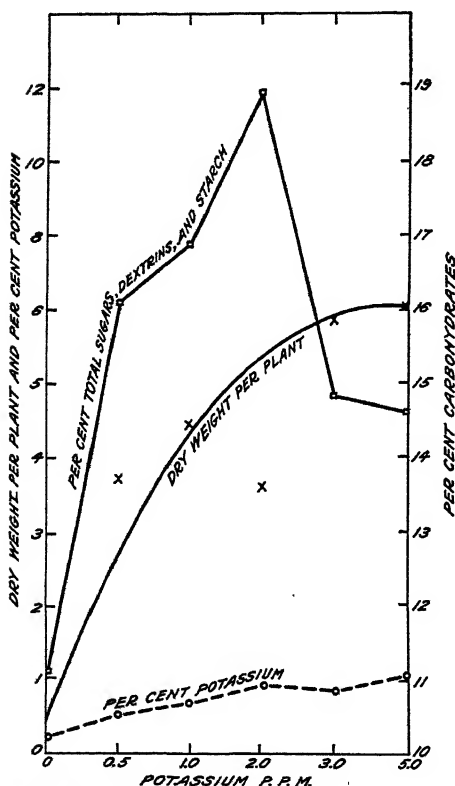


FIGURE 6.—Relations between the dry weight, potassium content, and carbohydrate constituents of Sudan grass when grown in nutrient solutions containing different amounts of potassium

fertilizer was no greater than in those receiving only nitrogen and phosphorus.

Data on soybean plants grown in sand-culture solutions are given in Table 5, A and B. The data shown in Table 5, A, are also presented in Figure 7. At both stages of plant development there is an increase in dry weight accompanying the increase of potassium in the nutrient solution upon which the plants were grown.

The sum of percentages of sugars and starch decreased in plants of the first collection with increase of potassium but increased in the second collection. This reverse change, like that in the case of oats (Table 1, A), may perhaps be accounted for by the presence of residual

potassium in the seed which supplied the plant in early development, whereas in later growth the potassium deficiency became pronounced, resulting in decreased carbohydrate synthesis.

TABLE 5.—Variations in carbohydrate constituents, dry matter, and potassium content of soybean plants grown on a balanced nutrient solution containing different amounts of potassium

A. GROWN IN SAND CULTURE

Jar No.	Date harvested	Potassium in solution	Plants per jar	Dry matter	Dry weight per 10 plants	Reducing sugars	Total sugars	Dextrins	Starch	Dextrins and starch	Sugars, dextrins, and starch	Hemicellulose	Potassium	Sugars, dextrins, and starch per plant
		P. p. m.	No.	P. ct.	Gm.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	Mgm.
132	Mar. 22	0	6	—	—	—	2.35	—	4.43	—	6.78	6.06	—	—
135	do	1	6	—	4.3	—	1.96	—	3.28	—	5.24	8.63	0.78	29.15
139	do	3	6	—	7.9	—	2.43	—	2.84	—	5.27	8.26	.81	41.39
143	do	5	6	—	9.6	—	2.79	—	2.11	—	4.90	8.96	1.00	50.59
147	do	10	6	—	13.0	—	—	—	—	—	—	—	.86	63.70
131	Apr. 12	0	6	—	4.8	—	1.50	—	.82	—	2.32	8.70	.40	11.13
136	do	1	6	—	10.8	—	1.70	—	1.18	—	2.88	8.06	.42	31.10
140	do	3	6	—	18.0	—	1.02	—	1.60	—	2.62	7.62	.62	47.16
144	do	5	6	—	26.0	—	1.30	—	3.41	—	4.71	8.61	.68	122.46
148	do	10	6	—	31.6	—	1.48	—	6.23	—	7.71	7.4	.86	243.63

B. GROWN IN SAND CULTURE WITH CONTINUOUS PERCOLATION OF NUTRIENT SOLUTION

149-50	-----	1	6	-----	7.1	-----	2.75	-----	2.22	-----	4.97	8.14	.80	35.28
151-52	-----	3	6	-----	11.4	-----	2.53	-----	3.29	-----	5.82	8.05	.78	66.40
153-54	-----	5	6	-----	12.9	-----	2.61	-----	3.60	-----	6.21	8.53	.85	80.10

C. GROWN IN WATER CULTURE

-----	0	8	20.83	4.7	1.653	2.480	0.799	2.148	2.947	5.427	11.119	-----	25.4
-----	0.5	8	15.90	14.8	1.210	1.772	.778	1.070	1.848	3.620	10.740	1.13	53.4
-----	1.0	8	15.00	13.0	1.040	1.489	.893	1.117	2.010	3.499	10.969	1.63	45.7
-----	2.0	8	14.55	17.6	.932	1.526	.974	1.185	2.159	3.685	11.792	1.77	64.9
-----	3.0	8	14.05	17.0	.852	1.476	1.061	.883	1.944	3.420	10.232	2.21	58.2
-----	5.0	8	14.70	19.3	.836	1.426	.762	1.059	1.821	3.247	11.113	1.97	62.6

* Sample was lost.

The data in Table 5, B, for soybeans grown in sand to which nutrient solutions were added by continuous percolation show the greatest dry weight per plant and the highest percentage of sugar and starch in the plants that received a nutrient solution containing 5 p. p. m. potassium.

The data for soybeans grown in water-culture solutions to which various increments of potassium were added are given in Table 5, C. The trend of the total dry weight per plant up to a concentration of 2 p. p. m. of potassium was to increase with the increase in percentage of potassium in the nutrient solution. Though good total growth was obtained, the plants were abnormally long and somewhat viney. The percentage of sugars and starch are more or less similar in plants grown at all potassium concentrations. Only those plants receiving no potassium showed a decided increase in these compounds. This can not be accounted for.

TABLE 6.—Variations in the carbohydrate constituents, dry matter, and potassium content of soybean plants when grown on Clarksville silt loam fertilized with different amounts of potassium

A. SOYBEANS GROWN ON FIELD SOIL IN 1927

Fertilizer	Sampling date	Part of plant	Plants			Reducing sugars	Total sugars	Dextrins		Starch	Dextrines and starch		Sugars, dextrins, and starch	Hemicellulose	Potassium	Sugars, dextrins, and starch in plants
			No.	P. ct.	Gm.			P. ct.	P. ct.		P. ct.	P. ct.				
Check.....	June 30..	Stem..	50	20.67	7.01	2.21	3.90	1.42	3.72	5.149	9.049	11.79	2.19			63.28
NP.....	do.....	do.....	50	22.00	10.20	2.44	3.82	1.35	3.00	4.352	8.172	12.72	1.77			83.35
NP K 150.....	do.....	do.....	50	19.32	10.73	1.74	3.04	1.42	3.22	4.649	7.689	13.33	2.24			82.50
NP K 300.....	do.....	do.....	50	18.00	12.70											
NP K 450.....	do.....	do.....	50	19.32	16.75	1.36	2.74	1.23	1.69	2.927	5.667	13.66	2.44			94.92
Check.....	do.....	Leaf..	50	25.79	5.00	.82	3.19	2.59	5.61	8.20	11.290	10.80	1.93			56.50
NP.....	do.....	do.....	50	22.75	6.58	.75	1.93	2.40	5.37	7.774	9.754	11.54	1.64			64.18
NP K 150.....	do.....	do.....	50	22.74	7.58	.97	2.05	2.51	6.04	8.555	10.605	11.40	1.97			80.38
NP K 300.....	do.....	do.....	50	22.00	10.07	.82	2.21	2.55	5.64	8.190	10.400	12.12	1.84			104.80
NP K 450.....	do.....	do.....	50	20.67	11.37	.89	2.29	2.59	4.01	6.600	8.890	10.54	2.00			102.21

B. SOYBEANS GROWN ON FIELD SOIL IN 1928

Check.....	Aug. 16.	Entire	100	20.75	16.081	1.699	2.616	1.45	4.391	5.849	8.465	10.03	1.38			136.11
NP.....	do.....	do.....	100	20.00	16.520	1.749	2.533	1.63	5.041	6.724	9.257	10.73	1.65			152.92
NP K 150.....	do.....	do.....	100	19.00	16.796	1.899	2.816	1.62	5.516	7.141	9.957	10.43	1.97			167.17
NP K 300.....	do.....	do.....	100	19.75	20.145	1.326	2.199	1.40	5.516	6.924	9.123	10.43	2.34			183.78
NP K 450.....	do.....	do.....	100	19.25	17.864	1.396	2.533	1.19	4.991	6.190	8.723	10.93	2.07			155.79
Check.....	Sept. 1.	do.....	40	20.5	11.00	1.53	2.61	1.35	5.116	6.466	9.078	12.16	1.56			249.59
NP.....	do.....	do.....	40	21.0	17.43	1.53	2.56	1.57	4.716	6.286	8.846	12.21	1.27			385.42
NP K 150.....	do.....	do.....	40	21.0	19.40	1.53	2.34	1.48	5.336	6.916	9.156	13.26	1.68			444.06
NP K 300.....	do.....	do.....	40	21.0	22.26	1.55	2.62	1.31	4.506	5.816	8.436	12.32	1.54			469.04
NP K 450.....	do.....	do.....	40	21.0	23.47	1.362	2.40	1.51	5.663	7.173	9.573	11.50	1.87			561.93

In 1927 samples of soybeans were collected from field plots fertilized with various amounts of potassium. The plants were separated into stem and leaves and analyzed. The data are given in Table 6, A. The plants grown on plots receiving the largest potassium application (450 pounds KCl per acre) had the largest total dry weight. The percentage of sugars and starch in the stems of these plants decreased with an increase in the amount of potassium added to the soil. These results are similar to those obtained from cornstalks. (Table 4, C.) The leaves of the soybeans from the various potassium soil treatments show practically no difference in the combined percentage of sugars and starch.

The analyses of soybeans collected in 1928 at two stages of development (Table 6, B) also show with the exception of the August 16 sample from the heaviest potassium application an increase in dry weight per plant with the increase in potassium fertilizer applied to the soil. In the first collection the total percentage of sugars and starch is slightly higher in plants grown on plots receiving the 150 pounds per acre KCl application; for the second collection, September 1, 1928, the results fluctuate and no definite relationship appears to exist.

The data for the sum of the total sugars, dextrins, and starch per plant of oats and soybeans grown in sand and for cotton, Sudan grass, and cowpeas grown in water are shown in milligrams in Figure 8. This figure shows that in so far as the total amounts of these substances

are concerned they are directly correlated with the highest percentage of potassium in the nutrient solution on which the plants grew.

DISCUSSION

The results of an earlier (2) investigation of plants grown in water cultures with potassium concentrations ranging from 1 to 5 p. p. m. showed that the largest percentage of potassium was taken up by the plants grown on the medium receiving the heaviest application of potassium. These results agree with those of other workers (5, 6, 7, 8, 9). The data further indicated that more potassium was taken from nutrient solutions containing large amounts of this ion than was actually required for the needs of the plant. Similar results were obtained in the present experiments, as shown by the total dry weights and percentages of potassium in plants. (Tables 1, B; 3, A; 4, A; and 5, C.) All these plants were grown in solutions in which the parts per million of potassium were kept as nearly constant as possible. Similar relations, however, of total dry weight to percentage of potassium in the plant under field soil conditions will be noted in Tables 2, A and C; 3, B and C; and 6, B.

This indicates that a greater percentage of potassium is taken up by the plant than is actually needed in its metabolism. These results have been noted in a previous article (2) and are in agreement with the findings of Hopkins (15), Lende (19), and Blair (8).

It will be noted from Tables 1 to 6 that in many instances there is no significant relationship between the potassium of a plant and the amount of sugars and starch present. Thus oats grown in sand cultures (Table 1, A, and fig. 1) to which increments of potassium were added, and harvested February 9, show no definite relation of total percentage of sugar and starch to the percentage of potassium in the plant. However, the data from analyses of plants harvested on March 10 and April 12 do show some significant relation in total percentage of sugars and starch to that of the higher percentages of potassium in the plant. This lack of correlation between sugars, starch, and potassium in the plants first harvested may have been due to the fact that the potassium in the seed had been sufficient to supply the plant to at least this stage of development. At the time of the earlier harvest the plants were approximately 6 inches high.

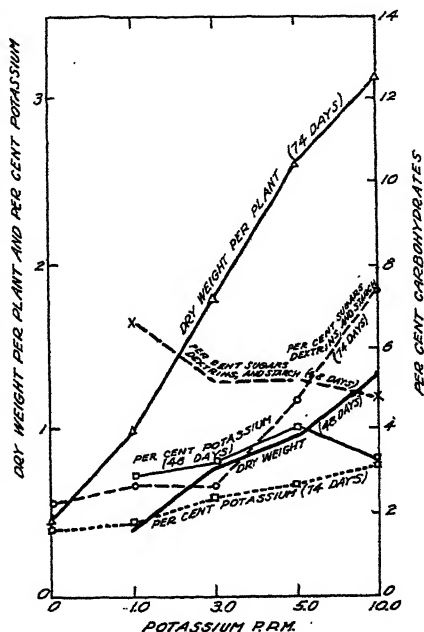


FIGURE 7.—Relations between the dry weight, potassium content, and carbohydrate constituents of soybean plants when grown in sand cultures containing different amounts of potassium

A similar relationship between the percentage of carbohydrates and the percentage of potassium in the plant may be noted in the case of soybeans. (Table 5, A, and fig. 7.) In this case, as in the oats, there is for the first analysis (March 22, 1927) a decrease up to 5 p. p. m. of potassium in the nutrient solution in the percentage of total sugars and starch with an increase in the percentage of potassium in the plant and for the second analyses (April 12, 1927) there is an increase. The same explanation given for the oats may hold true in the case of the soybeans. However, when the data for the oats

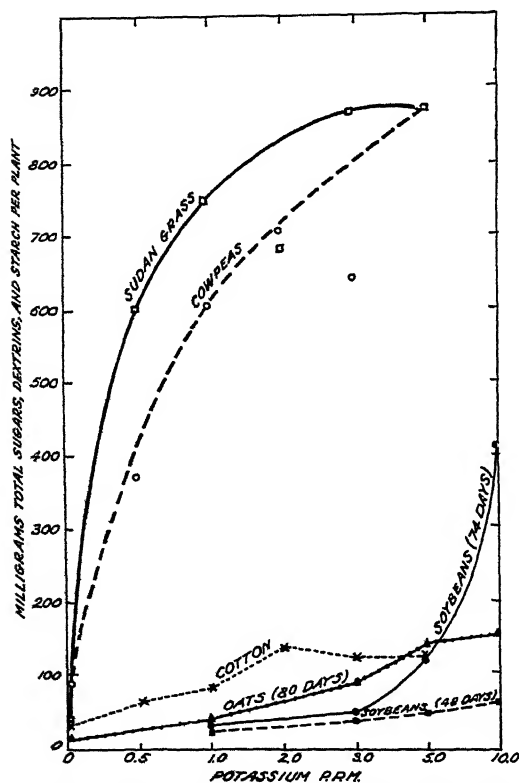


FIGURE 8.—Relations between the total sugars, dextrins, and starch of different plants when grown on nutrient solutions containing different amounts of potassium

(Table 1, B) and soybeans (Table 5, B) grown in sand, with continuous percolation of the nutrient solution, are examined it will be seen that the highest percentage of sugars and starch was obtained in the plants grown in nutrient solutions containing 3 p. p. m. of potassium, whereas in the case of soybeans there is a consistent increase in the carbohydrate compounds in plants up to 5 p. p. m. of potassium in the solution.

Figures 4, 5, and 6, for cowpeas, cotton, and Sudan grass grown in water-culture solutions to which the increments of potassium added over the potassium-free solution were 0.5, 1.0, 2.0, 3.0, and 5.0 p. p. m. and kept at these concentrations by renewing the amounts twice daily, show that there is a tendency for the percentage of sugar and

starch to decrease at about 2 to 3 p. p. m. of potassium. These results agree with the work of Stoklasa and Pitra (30), who grew barley in pot cultures with full fertilizers but with different quantities of potassium. They used the following amounts of KCl per jar: 0.5, 1.0, 1.5, and 3.0 gm. The percentage of starch in these plants is, respectively, as follows: 57.38, 63.52, 64.00, 61.20, and 57.68, showing that the greatest percentage of starch was obtained at about 0.5 and 1 gm. KCl per jar. These data indicate that when an active supply of potassium is present in the soil solution the plant takes up more than is actually necessary for its metabolism. It seems therefore, very probable that the greatest plant growth would not take

place at the maximum potassium concentration of the soil solution, but at some intermediate point. Similarly, the maximum percentages of sugars and starch do not necessarily occur in plants when the potassium concentration is greatest, as may be observed in these experiments, but rather at an intermediate point. In the work reported no plants were left growing to maturity, nor were any seeds analyzed, nor were any plants used in which special storage organs were concerned, such as roots or tubers. The nearest approach to such organs are the stems of the plants used.

Other investigators seem also to have found that there is not always a direct correlation between the potassium in the plant and sugar produced. Thus, Andriik (1) has noted that the proportion of potassium taken up by the plant to the quantity of sugar produced was not constant, but varied from 2.1 to 5.8 parts potassium to 100 parts sugar. Schneidewind (27), in experiments with potatoes on sand and with the use of different fertilizers, showed that the use of a 40 per cent potassium salt as an application in the spring caused a marked reduction in the starch content of potatoes. However, with potassium sulphate the starch content was increased. In the chemical investigations of Morse and Jones, as reported by Haskell (13), it was found that sugars were consistently lower in the wood of plants grown on soil treated with potassium chloride. Kraft (18) noted that potassium fertilizers alone heavily decreased the dry matter, starch, and protein content of potatoes, but increased the percentage of water and mineral matter and had an unfavorable action on flavor.

These investigations suggest that the type of fertilizers, the type of plants, and the nature of the environment play a large part in the response of the plant to potassium. The effect of temperature on the assimilation of potassium in the plant has been shown by Tottingham (32), and his results indicate that temperature may be an important factor in potassium assimilation. It is very possible that the above factors would greatly modify the potassium and carbohydrate relationship.

In order to correlate the total sugars and starch per plant with parts per million of potassium in the nutrient solution upon which the plants were grown, the total weight of sugars and starch per plant of cowpeas, Sudan grass, cotton, soybeans, and oats have been graphed in Figure 8. With the exception of the cotton plant, which shows a greater amount of total sugar and starch per plant at 2 p. p. m. of potassium, all other plants named show a greater quantity of these compounds at the highest concentration of potassium used in the nutrient solution. If these data are compared with the percentage composition of these same compound figures it will be seen that the largest percentage of these compounds was obtained when the nutrient solution contained approximately 2 or 3 p. p. m. of potassium. The relationship of total sugars and starch compounds per plant between plants grown on soils fertilized with potash and plants grown on soils not fertilized with potash is in harmony with data presented by Hall (11) and Stoklasa (29). However, it should be expected that as the dry weight per plant increases, the total sugars and starch should also increase, even though the percentage of these compounds remained the same. Hence the total amount of these compounds in the plants can not be used as a criterion by which to estimate the activity of potassium in their formation. If, on the other hand, the

percentage of these compounds is considered with that of potassium it will be noted that the maximum increase takes place, not at the greatest percentage of potassium in the plant but at an intermediate point.

It appears, therefore, that there is an optimum potassium concentration in the plant at which CO_2 assimilation is at its maximum and above or below which it again decreases. These optimum relationships no doubt are difficult to define. The most that can be said is that they are as complex for the potassium ion as for any other ion in the plant, and, similarly, that the optimum under one set of environmental conditions may be different from that under other environmental conditions.

SUCCULENCE

In a previous paper (17) it was shown that tomato plants grown on a nutrient solution containing a large amount of potassium were more succulent than plants grown on a nutrient solution containing a very small amount of potassium. The degree of succulence was determined by the percentage of dry matter of the two types of plants.

In the present investigation attention is again called to the greater percentage of dry matter in the plants receiving small amounts of potassium. Thus oats grown on sand cultures which received no or little potassium, as compared with cultures which received 10 p. p. m. potassium, show the following differences in percentages of dry matter (Table 1, A) in favor of the plants grown on the no-potassium solution for the March 10 collections and on the 3 p. p. m. solution for the April 12 collection: 9.8 per cent and 1.6 per cent, respectively. Similarly, plants grown in water cultures with 0 or 5 p. p. m. of potassium show the following differences in dry matter: Cowpeas, 2.1 per cent (Table 3, A); cotton, 3.6 per cent (Table 3, C); Sudan grass, 4.4 per cent (Table 4, A); and soybeans, 6.13 per cent (Table 5, C) in favor of plants grown on a no-potassium solution.

The plants collected from the field soils to which various amounts of potassium were applied also show differences in percentage of dry weight. Thus plants grown on soil which received no potassium, as compared with those grown on soil which received muriate of potassium at the rate of 450 pounds per acre, show the following differences in percentage of dry matter: Oats, 6 per cent (Table 2, A); cowpeas, first collection, 2.25 per cent, second collection, 1.5 per cent (Table 3, B); Sudan grass, 1.5 per cent (Table 4, B); and corn leaves, 6.5 per cent (Table 4, C) in favor of plants grown on soil poor in potassium.

The data presented show quite conclusively that high-potassium plants are more succulent than low-potassium plants. Whether this is due to the better utilization of the nitrogen in the plant through the direct action of potassium or to the function of potassium in the formation of the precursory carbohydrate compounds, or even to the action of potassium alone, is a matter of conjecture.

SUMMARY

The present investigation includes the determination of potassium, sugars, dextrin, starch, and hemicellulose in soybeans, cowpeas, Sudan grass, oats, sweetclover, and corn grown in soil, sand, and

water culture solutions to which various amounts of potassium were added.

From the results of the work on water and sand culture as well as on field soils to which increments of potassium were added in amounts of 0 to 5 p. p. m. potassium for water culture, 0 to 10 p. p. m. potassium for sand culture, and 450 pounds KCl per acre on field soil, it is evident that plants vary with respect to the amount of potassium needed for the production of normal growth. It was found in all this work that the largest plant growth was obtained at an intermediate application of potassium, which in water cultures would appear to be about 2 to 3 p. p. m. of potassium as evidenced by dry weight per plant.

When the percentage of sugars and starch in plants grown in culture solution were totaled it was found that the largest percentage of these compounds was obtained at 2 to 3 p. p. m. of potassium, which was below the concentration at which maximum absorption of potassium took place. These results show that more potassium is actually taken up by the plant than is needed for its use.

The relation between the percentage of potash and that of carbohydrate compounds fluctuates greatly, and it appears that a high percentage of sugars and starch is not necessarily associated with a high percentage of potassium in the plant.

If the total weight of sugars and starch per plant is compared with the percentage of potassium in the plant, it becomes evident that the correlation is good.

Dry weight per plant decreases with the increase of potassium in the plant, or with the amount of potassium in the nutrient solution. The indications are that as the amount of potassium in the nutrient solution increases the succulence of the plant also increases.

LITERATURE CITED

- (1) ANDERLIK, K., and URBAN, J.
1908. DER NÄHRSTOFFVERBRAUCH DER RÜBE IM VEGETATIONSJAHRE UND SEINE BEZIEHUNG ZUM ZUCKERGEHALT DER WURZELN. *Ztschr. Zuckerindus. Böhmen* 32: [559]-575.
- (2) BARTHOLOMEW, R. P., and JANSSEN, G.
1929. THE RELATION BETWEEN CONCENTRATIONS OF POTASSIUM IN CULTURE SOLUTIONS AND OPTIMUM PLANT GROWTH. *Soil Sci.* 27: 189-202, illus.
- (3) BLAIR, A. W.
1919. UTILIZING SOIL POTASH BY MEANS OF INTERMEDIARY CROPS. *Soc. Prom. Agr. Sci. Proc.* 39: 69-74.
- (4) DOWDING, E. S.
1925. THE REGIONAL AND SEASONAL DISTRIBUTION OF POTASSIUM IN PLANT TISSUES. *Ann. Bot. [London]* 39: [459]-474, illus.
- (5) FEST, F.
1908. ÜBER DEN ZEITLICHEN VERLAUF DER NÄHRSTOFFAUFNAHME UND TROCKENSUBSTANZPRODUKTION BEI DER BUSCHBONE UNTER VERSCHIEDENEN DÜNGUNGS- UND WITTERUNGSVERHÄLTNISSEN. *Jour. Landw.* 56: 1-47, illus.
- (6) FRAPS, G. S.
1912. THE ACTIVE POTASH OF THE SOIL AND ITS RELATION TO POT EXPERIMENTS. *Tex. Agr. Expt. Sta. Bul.* 145, 39 p., illus.
- (7) ———
1927. RELATION OF THE POTASH REMOVED BY CROPS TO THE ACTIVE, TOTAL, ACID-SOLUBLE, AND ACID-INSOLUBLE POTASH OF THE SOIL. *Tex. Agr. Expt. Sta. Bul.* 355, 33 p., illus.

- (8) FREAR, W., and ERB, E. S.
1918. CONDITION OF FERTILIZER POTASH RESIDUE IN HAGERSTOWN SILTY LOAM SOIL. *Jour. Agr. Research* 15: 59-81.
- (9) GODLEWSKI, E.
1923. SUR L'INFLUENCE DES ENGRAIS POTASSIQUES SUR LE DÉVELOPPEMENT ET LA COMPOSITION CHIMIQUE DES DIFFÉRENTES PLANTES CULTIVÉES. *Compt. Rend. Acad. Agr. France* 9: 404-412.
- (10) HAAS, P., and HILL, T. G.
1921-22. AN INTRODUCTION TO THE CHEMISTRY OF PLANT PRODUCTS. V. 1. ON THE NATURE AND SIGNIFICANCE OF THE COMMONER ORGANIC COMPOUNDS OF PLANTS. V. 2. METABOLIC PROCESSES. Ed. 3, 2 v., illus. London, New York, [etc.].
- (11) HALL, A. D.
1915. FERTILIZERS AND MANURES.
- (12) HARTWELL, B. L., and PEMBER, F. R.
1908. SODIUM AS A PARTIAL SUBSTITUTE FOR POTASSIUM. *R. I. Agr. Expt. Sta. Ann. Rpt.* 21: [243]-285, illus.
- (13) HASKELL, S. B.
1922. REPORT OF THE DIRECTOR. *Mass. Agr. Expt. Sta. Ann. Rpt.* 35, 25a p. (Pub. Doc. 31).
- (14) HOAGLAND, D. R., and MARTIN, J. C.
1923. EFFECT OF SALTS ON THE INTAKE OF INORGANIC ELEMENTS AND ON THE BUFFER SYSTEM OF THE PLANT. *Calif. Agr. Expt. Sta. Tech. Paper* 8, 26 p., illus.
- (15) HOPKINS, C. G., and AUMER, J. P.
1915. POTASSIUM FROM THE SOIL. *Ill. Agr. Expt. Sta. Bul.* 182, 10 p., illus.
- (16) JANSSEN, G.
1929. THE STUDY OF THE EFFECT OF DATE OF SEEDING OF WINTER WHEAT UPON SOME PHYSIOLOGICAL CHANGES OF THE PLANT DURING THE WINTER SEASON. *Jour. Amer. Soc. Agron.* 21: 168-200, illus.
- (17) ——— and BARTHOLOMEW, R. P.
1929. THE TRANSLOCATION OF POTASSIUM IN TOMATO PLANTS AND ITS RELATION TO THEIR CARBOHYDRATE AND NITROGEN DISTRIBUTION. *Jour. Agr. Research* 38: 447-465, illus.
- (18) KRAFT, A.
1920. DER EINFLUSS DER NÄHRSTOFFE AUF DIE QUALITÄT DER KARTOFFEL. *Arb. Forschungsinst. Kartoffelbau* 3 Heft: 1-11.
- (19) LENDE NJAA, J.
1912. LUKSBRUK AV FOSFORSYRE OG KALI. [PRESUMED LUXURY CONSUMPTION OF POTASSIUM AND PHOSPHORIC ACID.] *Meddel. Norske Myrselskap.* 10 aarg (5), p. [137]-201. ((Abstract) *Chem. Abs.* 8: 1322).
- (20) LINK, K. P., and TOTTINGHAM, W. E.
1923. EFFECTS OF THE METHOD OF DESICCATION ON THE CARBOHYDRATES OF PLANT TISSUE. *Jour. Amer. Chem. Soc.* 45: 439-447.
- (21) LOEW, O.
1903. THE PHYSIOLOGICAL RÔLE OF MINERAL NUTRIENTS IN PLANTS. *U. S. Dept. Agr., Bur. Plant Indus. Bul.* 45, 70 p.
- (22) MCCALL, A. G., and RICHARDS, P. E.
1918. MINERAL FOOD REQUIREMENTS OF THE WHEAT PLANT AT DIFFERENT STAGES OF ITS DEVELOPMENT. *Jour. Amer. Soc. Agron.* 10: 127-134, illus.
- (23) MATOUŠEK, A.
1914. BEITRAG ZUR KENNTNIS DER LOKALISATION DER KALIVERBINDUNGEN IN DER ZUCKERÜBE UND IHRER PHYSIOLOGISCHEN BEDEUTUNG. *Ztschr. Zuckerindus. Böhmen* 38: [235]-251, illus.
- (24) MURNEEK, A.
1926. EFFECTS OF CORRELATION BETWEEN VEGETATIVE AND REPRODUCTIVE FUNCTIONS IN THE TOMATO (*LYCOPERSICON ESCULENTUM* MILL.). *Plant Physiol.* 1: 3-56, illus.
- (25) PARKER, F. W.
1927. SOIL PHOSPHORUS STUDIES: III. PLANT GROWTH AND THE ABSORPTION OF PHOSPHORUS FROM CULTURE SOLUTIONS OF DIFFERENT CONCENTRATIONS. *Soil Sci.* 24: 129-146.

- (26) REED, H. S.
1907. THE VALUE OF CERTAIN NUTRITIVE ELEMENTS IN THE PLANT CELL. *Ann. Bot. [London]* 21: [501]-543, illus.
- (27) SCHNEIDEWIND, [W.]
1919. WIRKUNG UND ANWENDUNG DER NEUEN DÜNGEMITTEL. *Jahrb. Deut. Landw. Gesell.* 34: 305-313.
- (28) SEISSL, J., and GROSS, E.
1902. UEBER DEN KALI- UND PHOSPHORSÄUREGEGHALT DER BLATTASCHEN VERSCHIEDEN STÄRKEREICHER KARTOFFELSORTEN. *Ztschr. Landw. Versuchsw. Österr.* 5: 862-875.
- (29) STOKLASA, J.
1912. IST DAS KALIUM AN DEM AUF-UND ABBAU DER KOHLENHYDRATE BEI HÖHEREN PFLANZEN BETEILIGT? *Ztschr. Landw. Versuchsw. Österr.* 15: 711-736.
- (30) ——— and PITRA, J.
1901. UEBER DIE WIRKUNG DER KALISALZE AUF DIE ENTWICKLUNG DER GERSTE. *Ztschr. Landw. Versuchsw. Österr.* 4: [567]-582, illus.
- (31) THATCHER, R. W.
1921. THE CHEMISTRY OF PLANT LIFE. 268 p. New York.
- (32) TOTTINGHAM, W. E.
1926. TEMPERATURE EFFECTS IN THE METABOLISM OF WHEAT. *Plant Physiol.* 1: 307-336, illus.
- (33) WILFARTH, H., and WIMMER, G.
1902. DIE WIRKUNG DES KALIUMS AUF DAS PFLANZENLEBEN NACH VEGETATIONSVERSUCHEN MIT KARTOFFELN, TABAK, BUCHWEIZEN, SENF, ZICHORIEN UND HAFER. *Arb. Deut. Landw. Gesell.* 68, 106 p., illus.

A STUDY OF THE MOSAIC DISEASE OF CRUCIFERS ¹

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INTRODUCTION

Mosaic diseases of plants have become increasingly serious during the last quarter of a century, or else the damage done by them has become more fully recognized. In the course of a general investigation of diseases of cruciferous crops the writer has given particular attention to the mosaic disease affecting them. The trouble was observed in rutabaga fields on Long Island, and, in the beginning, there was a question as to whether a disease of cauliflower called whip-tail might not be a mosaic. The malnutritional nature of the whip-tail disease has been established,² however, and it is intended here to record the results of the investigations of the true cruciferous mosaic.

Literature references to the cruciferous mosaic in this country are limited to simultaneous publications by Gardner and Kendrick³ and Schultz⁴ recording its appearance and the infectious nature of the disease. Schultz found the mosaic intertransmissible between turnip, mustard, and Chinese cabbage, but the potato and morning-glory were not infected. He also found that mustard seed from mosaic plants produced healthy seedlings. Gardner and Kendrick reported that the disease was easily transmitted from turnip to turnip but not from turnip to radish. Gram⁵ states that in Denmark the disease was observed on turnips in a number of localities and that different varieties of turnips, rutabagas, Raphanus, and *Sinapsis arvensis* were susceptible.

The chief object of the investigation reported in this paper was to determine whether the mosaic disease known to attack certain crucifers was potentially a serious trouble of any of the three important cruciferous crops grown on Long Island, N. Y.; that is, cabbage, cauliflower, and Brussels sprouts.

RELATION OF DISEASE TO SEED

In the fall of 1923 the writer selected 20 rutabagas from a healthy field and a like number of mosaic-affected roots from a near-by field in which about 5 per cent of the plants were diseased. These two lots were stored separately and during mid-March of the following year 12 of the best roots from each lot were set out in the greenhouse. For various reasons 2 plants of each lot were later removed, but the remaining 18 plants grew and bore seed. It was observed

¹ Received for publication May 8, 1929; issued February, 1930.

² CLAYTON, E. E. INVESTIGATIONS OF CAULIFLOWER DISEASES ON LONG ISLAND. N. Y. State Agr. Expt. Sta. Bul. 506, 15 p., illus. 1924.

³ GARDNER, M. W., and KENDRICK, J. B. TURNIP MOSAIC. Jour. Agr. Research 22: 123-124, illus. 1921.

⁴ SCHULTZ, E. S. A TRANSMISSIBLE MOSAIC DISEASE OF CHINESE CABBAGE, MUSTARD AND TURNIPS. Jour. Agr. Research 22: 173-178, illus. 1921.

⁵ GRAM, E. MOSAIKSYGE HOS KORSHLOMSTREDE. [MOSAIC IN CRUCIFERS.] Dansk Frøavl. [København] 8: 41-42. 1925. [(Abstract) Bot. Abs. 15: 782. 1926.]

that the mosaic roots were slower to develop seed stalks than the healthy, and, as the leaves appeared, all those from the mosaic roots were mottled. The mottling was most conspicuous on the older leaves, the younger growth in each case appearing healthy. The mosaic roots produced smaller tops than the healthy, and many of the blossoms dropped without developing pods. The average weight of dry seed produced by the plants grown from mosaic roots was 0.74 ounce, while the same number of plants from healthy roots averaged 1.85 ounces. The difference was chiefly due to the smaller number of pods on the mosaic plants. Counts and measurements showed that the individual pods on the mosaic plants were almost as large, and that the individual seeds were quite as large, as those on healthy plants, also, that the number of seeds per pod was almost the same on mosaic and healthy plants. The seed from healthy and diseased plants was kept separate and sown in three different localities in 1924. No mosaic appeared in two of these plantings all season and in the other only a few cases were observed toward the end of the season. The results indicate, therefore, that the disease is not carried in rutabaga seed, thus confirming the findings of Schultz with mustard.

HOST RANGE

The susceptibility to mosaic of turnips, rutabagas, mustard, and Chinese cabbage appeared to be well established, but the reaction of the more important crucifers, cabbage, cauliflower, and Brussels sprouts, was not known; consequently, inoculation experiments were undertaken with these crops. The cabbage aphid (*Brevicoryne brassicae* (L.)) was found to be exceptionally effective as an insect carrier and was used in most of the inoculation work. The green peach aphid (*Myzus persicae* (Sulz.)) was tried out twice with unsatisfactory results. Inoculation by hand consisted in rubbing crushed tissues from diseased plants into the leaves of healthy plants in such a way as to injure the midrib. This method was highly successful with the more susceptible host plants but not with those possessing a degree of resistance. The incubation period varied from three to more than five weeks and was shortest with the most susceptible host plants. Diseased rutabagas were the usual source of inoculum, and these proved very satisfactory. Rutabagas are easily infected and if kept in a cool place the diseased plants continue to make fair leaf growth. The cabbage aphid also multiplies freely on this host.

INOCULATION RESULTS IN THE GREENHOUSE

CHINESE CABBAGE (*BRASSICA CHINENSIS* L.).⁶—The plants were readily infected and showed inconspicuous mottling. At temperatures between 70° and 80° F. diseased plants were much stunted and developed streak symptoms. This plant was classed as highly susceptible.

MUSTARD, CULTIVATED WHITE (*BRASSICA ALBA* RABENH.) AND **WILD BLACK** (*NIGRA* KOCH).—The seed of the cultivated variety was purchased and that of the wild variety was collected from the fields. The plants of both were readily infected and reacted much like the

⁶ The nomenclature followed is that used in Bailey's Manual of Cultivated Plants. BAILEY, L. H. *MANUAL OF CULTIVATED PLANTS*. 851 p., illus. New York. 1924.

Chinese cabbage. The leaves showed faint mottling and, at higher temperatures, plants were yellow and much stunted, usually developing streak. The mustards, while slightly less susceptible than the Chinese cabbage, were still highly susceptible.

FLAT TURNIP (*BRASSICA RAPA* L.).—The plants were readily infected and developed pronounced leaf mottling. At high temperatures diseased plants showed yellowing and stunting, but never developed streak. This plant was easily infected and susceptible, but was not as severely injured by the disease as mustard and Chinese cabbage.

RUTABAGA OR SWEDE (*BRASSICA NAPOBRASSICA* MILL.).—The behavior of this plant was similar to that of the flat turnip.

RAPE (*BRASSICA NAPUS* L.).—Several plants were inoculated in early November, 1925, with apparently negative results. They were held over winter, and one of them, about February 1, when flower shoots were forming, suddenly developed mottling that affected all of its leaves, and from then on it continued to show marked mosaic symptoms. The plants were growing in 3-gallon crocks and were very large at this time. The next fall six rape plants were inoculated and five showed distinct mosaic symptoms in four weeks; so the reason for the 3-months' incubation period of the previous year is not clear. In addition to mottling, the infected plants showed moderate stunting. The rape appeared to be somewhat less susceptible than the white turnip or rutabaga to which it is closely related.

BRUSSELS SPROUTS (*BRASSICA OLERACEA* L. VAR. *GEMMIFERA* ZENKER).—The early attempts to infect these plants were unsuccessful. As soon as the cabbage aphid was used as the inoculating agent, however, positive results were obtained, though the symptoms developed slowly. Occasional Brussels sprouts plants showed the usual type of mosaic mottling, but the great majority developed round yellow blotches that were scattered rather evenly over the leaves and were not raised. Later the tissues around the edges of the yellow areas died in a way that suggested the ring-spot disease of tobacco. Infected plants held at higher temperatures were stunted by the disease. At lower temperatures it was common for mosaic plants, after showing mosaic symptoms in half a dozen leaves, to recover and produce new leaves of normal appearance. None of the plants previously discussed have ever been observed to recover once they were infected, though the injury they suffered from the disease depended in part on environmental conditions.

The Brussels sprouts were distinctly more resistant to mosaic than the turnip or rape or any of the plants higher in the list.

CAULIFLOWER (*BRASSICA OLERACEA* L. VAR. *BOTRYTIS* L.).—As in the case of Brussels sprouts, early attempts to inoculate cauliflower failed but later efforts were successful. The symptoms that developed were similar to those on Brussels sprouts, with yellow spots scattered over the otherwise normal-appearing green leaf. However, in the case of the Brussels sprouts, the tissues around the edges of the spots died, and this did not occur in the cauliflower. Infected plants when placed under cool temperature conditions usually produced new leaves that showed no mosaic and, like the Brussels sprouts, outgrew the disease. During the course of the work a number of pure lines of cauliflower were inoculated by rubbing. In all lines but one, an occasional plant only developed mosaic symptoms and then of the usual mild character. Five plants of this one line were infected

and all showed the marked mottling and distortion of leaves such as occurs in turnips, indicating the possibility of mosaic-susceptible strains of cauliflower. The commercial cauliflower, however, was more resistant than the Brussels sprouts.

CABBAGE (*BRASSICA OLERACEA* L. var. *CAPITATA* L.).—Many plants were inoculated, but none developed positive symptoms of mosaic. A number of plants that developed doubtful symptoms were tested by using leaf tissues from them for back inoculating healthy rutabaga plants. The results of these tests were negative; hence, on the basis of the inoculation work conducted in the greenhouse, the cabbage was rated as immune to the cruciferous mosaic.

FIELD EXPERIMENTS AND OBSERVATIONS

On May 27, 1926, two beds were prepared and cauliflower, cabbage, Brussels sprouts, and rutabaga seeds were sown in separate rows. Two weeks later one bed was inclosed with cheesecloth and the plants were inoculated by allowing cabbage aphids taken from mosaic rutabagas to feed upon them. The aphids multiplied, and after 10 days all were killed with nicotine. On July 1, lots of 20 plants were set out from each seed-bed row from the inoculated bed and 4 from the check.

By August 20 the inoculated rutabagas were hardly a fifth the size of the uninoculated, and mosaic symptoms were well developed. The inoculated Brussels sprouts also had developed mosaic with the characteristic yellow leaf blotches. The inoculated cauliflower plants were not all growing as rapidly as the uninoculated, but none showed mosaic leaf symptoms. The inoculated and uninoculated cabbage plants were growing equally well with no visible differences. Typical mosaic symptoms on rutabaga, Brussels sprouts, and cauliflower leaves are shown in Figure 1.

By September 10, all 20 uninoculated rutabaga plants had contracted mosaic, though they were not yet stunted by the disease. All 20 of the inoculated Brussels sprouts plants showed the yellow mosaic spots; and around the borders of these were the circular rings of dead tissue peculiar to the disease on this plant. Two of the uninoculated Brussels sprouts now showed mosaic symptoms on the leaves, but whereas the 20 inoculated in the bed were much dwarfed these two later infected plants were still of normal size. Of the 20 inoculated cauliflower, 11 showed faint mosaic mottling at this time and these were all distinctly dwarfed. The remaining 9 inoculated were growing normally as were all 20 uninoculated. Three of the 20 inoculated cabbages showed faint markings that might indicate mosaic.

On October 1 the field experiment was concluded, and healthy greenhouse-grown rutabaga plants were inoculated by rubbing the leaves with crushed tissues from mosaic field-grown rutabaga, Brussels sprouts, and cauliflower plants. Three to five of the diseased field plants of each kind were selected and also the three cabbage plants that had shown possible mosaic symptoms. Two greenhouse rutabaga plants were inoculated with tissue from each field plant. Check pairs of rutabaga plants were inoculated with crushed tissues of healthy rutabaga, Brussels sprouts, cauliflower, and cabbage, these plants all being selected some distance away from any mosaic.

All six greenhouse rutabagas inoculated with tissues from mosaic field rutabagas developed mosaic. Of 10 greenhouse rutabagas,

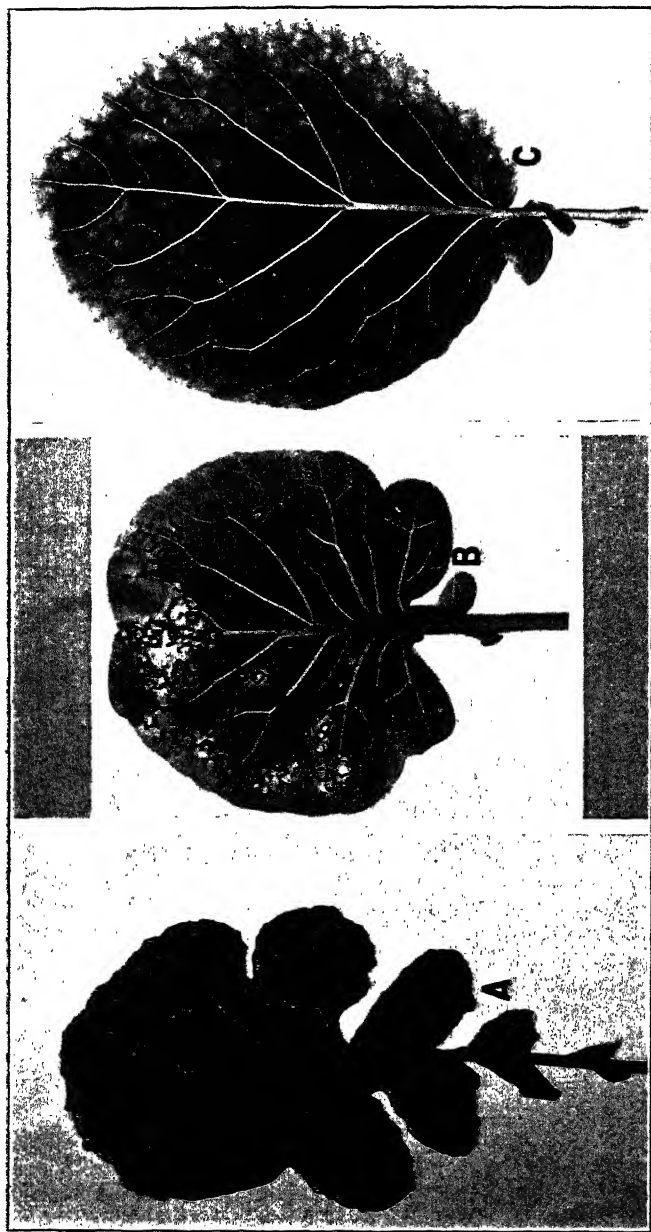


FIGURE 1.—A, Mosaic symptoms on a rutabaga leaf. Affected leaves show yellowing and are sometimes blistered; the mosaic pattern of light and dark patches is distinct and occasionally small necrotic areas develop. B, Mosaic symptoms on a Brussels sprouts leaf. Scattered yellow blotches appear on these leaves and somewhat later the tissues die at the edges of the spots forming "horseshoes." C, Mosaic symptoms on a cauliflower leaf. The mottling greatly resembles that appearing on Brussels sprouts leaves at an early stage of the disease; the numerous yellow blotches are very slightly raised and there is no leaf distortion nor necrosis.

representing 5 mosaic field cauliflower plants, 8 developed mosaic and 2 remained healthy. The same numbers with Brussels sprouts gave 6 mosaic and 4 healthy. The 6 rutabaga plants inoculated from the 3 cabbages gave 2 mosaic (both inoculated from the same cabbage plant) and 4 healthy.

Mention has been made of the recovery of Brussels sprouts and cauliflower plants under greenhouse conditions. In the previous field experiment the Brussels sprouts plants that showed mosaic symptoms during the summer and were severely stunted began to improve about September 1, and by October 1 fully two-thirds of them had developed new and vigorously growing tops that appeared healthy. The same thing has been observed many other times in the field, and experienced growers, when shown diseased plants, have predicted that they would start to grow as soon as cool weather came. Mosaic cauliflower plants showed the same ability to recover under field conditions, but mosaic rutabagas, while making a better growth during cool weather, have always shown unmistakable symptoms of the disease. Another interesting field phenomenon that occurs rarely with either Brussels sprouts or cauliflower is the localization of the mosaic symptoms in the leaves on only one side of the plant.

OCCURRENCE OF DISEASE IN THE FIELD

The flat white turnip, rutabaga, Brussels sprouts, cauliflower, and cabbage are the common cultivated crucifers grown on Long Island and the black mustard is a prevalent weed. Mosaic has not been observed on white turnips. These are usually grown as a catch crop late in the summer in the market-gardening districts where the disease appears not to be established. Mosaic is common in the rutabaga fields of the Port Jefferson section, but has never caused serious loss. This is correlated with the fact that most of the infections occur late in the season and that the total infection in rutabaga fields has rarely exceeded 10 per cent. In Brussels sprouts fields in the East Marion section the mosaic disease has been commonly observed, the infection ranging from 5 per cent to a trace. In this locality black mustard is a common weed in and about Brussels sprouts fields and mosaic-diseased plants are frequently seen. Since the Brussels sprouts is a biennial and the mustard an annual, it appears likely that this is a case of a weed host contracting mosaic from a cultivated host. Many Brussels sprouts plants survive the winter in the fields. Mosaic is rare in cauliflower fields and 0.75 per cent is the maximum amount ever observed in a field.

No evidence of mosaic has ever been observed in cabbage fields, but the following instance points to cabbage as a possible carrier of the disease. A farmer who grew seed cabbage found his plants heavily infested with cabbage aphids at the time of cutting. As soon as the seed was harvested, the land was plowed and set with Brussels sprouts. A month later there was a general cabbage-aphid infestation, and over 80 per cent of the plants by actual count showed mosaic symptoms. This is about 15 times the maximum mosaic ever observed by the writer in any ordinary field of Brussels sprouts. The previous crop of aphid-infested cabbage appears to provide the only explanation of this outbreak of the disease, and the circumstantial evidence favors the idea that cabbage, while highly resistant to mosaic, may serve as a carrier.

DISCUSSION OF RESULTS

Since Brussels sprouts and cabbage are both hardy enough to live over winter unprotected on Long Island and in other localities where winters are not too severe, they offer a possible explanation of the method whereby the cruciferous mosaic carries over. At present no perennial weed hosts are known, and the tests with seed from mosaic rutabaga plants showed that the disease was not seed borne.

The cruciferous mosaic was found to be very responsive to environmental conditions and to injure the host plants most at high temperatures. (Fig. 2.) The susceptible Chinese cabbage and mustard developed the virulent streak form of mosaic when grown at a temperature of



FIGURE 2.—Relation between temperature and growth of healthy and mosaic-infected Chinese cabbage plants: A, Healthy (a) and infected (b) plants grown at 55° to 65° F.; B, healthy (a) and infected (b) plants grown at 70° to 80° F.

70° to 80° F. On the other hand, the less susceptible Brussels sprouts and cauliflower recovered completely when held at temperatures of 55° to 65°. The rapidity of field spread and the speed with which mosaic symptoms appeared varied directly with the susceptibility of the host plants, being greatest in both cases with the more susceptible. The cabbage aphid proved to be a very effective agent of dissemination in that it infected plants with great certainty. In the field, however, this insect is a very slow traveler. To what extent the green peach aphid is concerned in the spread of cruciferous mosaic is not certain. Schultz⁷ reported that the insect was a good agent of dissemination, but in these tests it was very much less effective than the cabbage aphid.

⁷ SCHULTZ, E. S. Op. cit.

Under Long Island conditions, with the crucifers grown as fall crops when the weather is cool, and with the more resistant of the crucifers only being cultivated, there is no reason to believe that this mosaic disease will ever be a serious problem. In the same locality it would appear likely that susceptible hosts such as Chinese cabbage and mustard if grown during midsummer might be seriously injured and it has been proved by actual test that such is the case.

SUMMARY

The mosaic disease of crucifers is a common but not serious disease of rutabagas on Long Island, N. Y. The disease has also been observed on Brussels sprouts, cauliflower, and black mustard.

Roots from diseased rutabagas held over winter threw up seed stalks which showed mosaic symptoms but matured seed. This seed was of normal appearance and the crop grown from it was healthy.

White and black mustards, Chinese cabbage, turnips, rutabagas, and rape were found to be susceptible hosts. Brussels sprouts and cauliflower, although susceptible, were not easily infected. Cabbage was either highly resistant or immune to the disease with some evidence that it may serve as a carrier.

Symptoms varied widely with the different host plants and also with environmental conditions.

At 70° to 80° F. the mustards and Chinese cabbage developed streak and the other plants were much stunted. At 55° to 65° even the more susceptible hosts made a fair development, while mosaic Brussels sprouts and cauliflower often ceased to show mosaic symptoms and grew normally. Under field conditions where these crops are sown in summer and develop during the fall it is usual for diseased Brussels sprouts and cauliflower to begin to recover about September 1.

It is conceded that cruciferous mosaic will continue to be a minor disease on Long Island because of the natural resistance of the most important economic crucifers, cabbage, cauliflower, and Brussels sprouts and also because these crops are grown during the cool weather of fall, whereas the disease develops best at high temperatures.

THE INSENSIBLE LOSS IN BODY WEIGHT OF CATTLE¹

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INTRODUCTION

It is a fact of common knowledge that the total weight of the gaseous substances coming from the animal body always exceeds the weight of the gaseous intake; and the resulting insensible loss (often designated insensible perspiration) is one of the several factors responsible for the daily changes in the live weight of an animal, others being the quantities of food and water consumed and the weights of the visible excreta eliminated.

This subject has been extensively investigated with reference to human beings, and a historical account of such studies is given by Benedict and Root.³ Although many factors affecting the insensible loss are recognized, Benedict and his coworkers⁴ have found, in recent comprehensive experiments with human beings, that there exists a very close relationship between the insensible loss and the metabolic level. These researches have led the authors to the conclusion that the metabolism of human beings may be predicted with reasonable accuracy from the insensible loss, carefully determined under standard conditions.

Does a similar relationship exist in the case of cattle? Benedict and Ritzman⁵ have recently reported data obtained with steers bearing on this question. These authors have found the evidence "sufficient to conclude that the most potent factor in determining the magnitude of the insensible perspiration is the general nutritive plane or the metabolic level. In other words, the insensible loss probably is closely correlated with the total 24-hour metabolism of the animal at the time the insensible loss is measured." The authors call attention to the fact that the proof of the latter conclusion is lacking, since no simultaneous measurements of the 24-hour metabolism and of the insensible loss were made. They express, however, the belief that "the prediction of the total daily metabolism of steers may

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² The author is gratefully indebted to Dr. E. B. Forbes, director of this institute, for the encouragement received from him while preparing this paper and for the helpfulness of his criticisms. Acknowledgment is also made of the valuable assistance of H. H. Williams in checking some of the calculations.

³ BENEDICT, F. G., and ROOT, H. F. INSENSIBLE PERSPIRATION: ITS RELATION TO HUMAN PHYSIOLOGY AND PATHOLOGY. *Arch. Int. Med.* 38: 1-35, illus.

⁴ BENEDICT, F. G. BASAL METABOLISM: THE MODERN MEASURE OF VITAL ACTIVITY. *Sci. Mo.* 27: 5-27, illus. 1928.

— and BENEDICT, C. G. THE NATURE OF INSENSIBLE PERSPIRATION. *Natl. Acad. Sci. Proc.* 13: 364-369. 1927.

— and BENEDICT, C. G. PERSPIRATIO INSENSIBILIS: IHR WESEN UND IHRE URSACHEN. *Biochem. Ztschr.* 186: [278]-312, illus. 1927.

— and ROOT, H. F. *Op. cit.*

⁵ BENEDICT, F. G., and RITZMAN, E. G. THE METABOLISM OF THE FASTING STEER. p. 66-75. 1927. Washington, D. C. (Carnegie Inst. Wash. Pub. 377.)

actually be made with close approximation if the insensible loss, under controlled conditions of temperature, is accurately known."

An examination of the experimental records of this institute revealed the fact that in a large number of experiments with steers and cows data were available for the determination of the insensible loss, under controlled conditions, in the calorimeter, for periods during which the heat production was measured, and that data were also available for determining this loss in digestion trials under barn conditions.

The present paper, therefore, is devoted to computations of this insensible loss, and to a discussion of certain conditions of environment, the coat of hair of the animal, and the plane of nutrition, as they affect these values. The quantitative relation between the level of feeding and the insensible loss and the value of the latter as a basis for predicting the heat production are considered in a subsequent paper.⁶

CONDITIONS PREVAILING IN THE CALORIMETRIC EXPERIMENTS

In the metabolism experiments the animals were weighed on bullock scales (sensitive to 0.2 kgm.) immediately before entering the calorimeter and at the end of the calorimetric period. This period was 53 hours in length in the early experiments and from 85 to 87 hours in most of the more recent experiments. One calorimeter period considered, however (experiment 238, steer 36, period 12), was 111 hours in length. Records were also kept of the weights of feed and water consumed and of feces and urine eliminated during the period spent by the animal in the calorimeter. Data permitting the determination of the insensible loss were found available for 77 calorimetric periods, of which 58 were with steers, 11 with dry cows, and 8 with lactating cows.

The number of individual animals used as subjects in the 77 calorimetric experiments was 16, of which 4 were cows, and 12 were steers. The average live weights of the animals varied from 311 to 642 kgm.

The feed consumption in these experiments (except four fasting periods) varied between 392 and 2,268 gm. of dry matter per 100 kgm. of live weight per day. In 18 periods the rations consisted of roughage only, while in 55 periods they consisted of roughage and grain.

The temperature and the rate of ventilation were maintained uniform in the calorimeter during each of the experimental periods.

COMPUTATION OF THE INSENSIBLE LOSS OF BODY WEIGHT FROM THE LIVE WEIGHTS AND WEIGHTS OF FOOD, WATER, AND EXCRETA

The computation of the daily insensible loss from the daily live weights, the weights of the food and water consumed, and the weight of visible excreta is extremely simple, as will be seen from the following example: A steer weighed at 6 a. m. on October 1, 359.1 kgm. On October 2 at 6 a. m. he weighed 361.0 kgm. Between 6 a. m. October 1, and 6 a. m. October 2, he drank 16.1 kgm. of water, ate 6.51 kgm. of feed, and voided 6.69 kgm. of feces and 5.92 kgm. of

⁶ KRIS, M. QUANTITATIVE RELATIONS OF THE DRY MATTER OF THE FOOD CONSUMED, THE HEAT PRODUCTION, THE GASEOUS OUTGO, AND THE INSENSIBLE LOSS IN BODY WEIGHT OF CATTLE. *Jour. Agr. Research* 40: pp. 283-295.

urine. Had it not been for the insensible loss the steer's weight at 6 a. m. on October 2 would have been $359.1 + 16.1 + 6.51 - (6.69 + 5.92) = 369.1$ kgm. The difference between 369.1 and 361.0—that is, 8.1 kgm.—is the insensible loss in body weight, and represents the sum of the weights of the water vapor, the carbon dioxide, and the methane produced, minus the weight of the oxygen consumed. Stated in different words, this insensible loss is made up of the carbon and the hydrogen of the fat, the proteins, and the carbohydrates oxidized in the body; the incompletely oxidized products (CH_4) escaping from the animal as a result of carbohydrate fermentation in the intestinal tract; and the water vaporized through the lungs and the skin. The latter factor usually makes up the greater portion of the total insensible loss.

The example given above is illustrative of the method of computing the daily insensible loss in the body weight of steers. The same method of computation is applicable to cows, except that in the case of lactating cows the weight of the daily milk produced should be properly taken into account as one of the egesta.

The average daily insensible loss for a period of several days can be obtained by either of the following procedures: (1) The insensible loss is computed for each day, the values obtained then being averaged; or (2) the total insensible loss for the period is computed, this loss being divided by the number of days represented.

For the computation of the average insensible loss by the first method the daily live weights, taken at the same time each day, must be known. The second method involves the use of the weights of the animal taken only at the beginning and end of the period. The same results are obtained by both methods, although the second method is much the shorter.

The daily insensible loss of the body weight of the animal in each of the calorimeter periods obtained by using the live weights and weights of foods, water, and excreta is given in Table 1. These values are referred to as the observed insensible loss to distinguish them from another set of values, also given in Table 1, which were obtained by a computation involving the use of the respiratory products, these latter values serving as checks on the former.

TABLE 1.—A comparison of the directly observed insensible loss in body weight of cattle with that computed from the respiratory products

Experiment and animal No.	Period No.	Length of calorimeter period	Insensible loss per day in calorimeter		Difference	
			Observed loss in weight	Computed from respiratory products	Computed minus observed	In per cent of observed
Experiment 240:		Hours	Kgm.	Kgm.	Kgm.	Per cent
Steer 60.....	1	85.5	3.3	3.7	+0.4	+12.1
57.....	2	86.5	4.5	4.6	+1.1	+12.2
60.....	3	86.5	4.9	5.5	+1.6	+12.2
57.....	4	87.0	6.3	6.8	+1.5	+7.9
60.....	5	86.0	9.5	9.1	-4	-4.2
57.....	6	86.0	10.4	10.9	+1.5	+4.8
57.....	8	86.5	12.3	13.3	+1.0	+8.1
60.....	9	86.5	10.6	11.5	+1.9	+8.5
57.....	10	86.5	4.0	4.3	+3	+7.5
60.....	11	86.5	3.5	3.4	-1	-2.9
57.....	12	86.0	6.4	6.1	-3	-4.7
60.....	13	86.0	6.1	6.0	-1	-1.6

TABLE 1.—A comparison of the directly observed insensible loss in body weight of cattle with that computed from the respiratory products

Experiment and animal No.	Period No.	Length of calorimeter period	Insensible loss per day in calorimeter		Difference	
			Observed loss in weight	Computed from respiratory products	Computed minus observed	In per cent of observed
Experiment 238:		Hours	Kgm.	Kgm.	Kgm.	Per cent
Steer 47.....	1	87.0	9.4	8.6	-.8	-8.5
36.....	2	87.0	9.4	8.9	-.5	-5.3
47.....	3	87.0	8.6	8.4	-.2	-2.3
36.....	4	87.0	7.8	8.3	+.5	+6.4
47.....	5	87.0	4.9	4.1	-.8	-16.3
36.....	6	87.0	5.0	4.0	-1.0	-20.0
47.....	7	87.0	6.0	5.6	-.4	-6.7
36.....	8	87.0	5.8	5.7	-.1	-1.7
36.....	10	87.0	6.7	7.2	+.5	+7.5
36 ^a	12	111.0	3.5	^b 3.2	-.3	-8.6
Experiment 237:						
Steer 47.....	2	85.0	5.1	6.7	+1.6	+31.4
254.....	3	85.5	4.0	4.0	0	0
47.....	4	85.0	5.9	5.9	0	0
254.....	5	85.5	6.2	5.8	-.4	-6.5
47.....	6	85.5	6.5	6.1	-.4	-6.2
254.....	8	85.0	6.7	6.2	-.5	-7.5
36.....	10	86.0	5.2	5.6	+.4	+7.7
47.....	11	85.5	5.0	5.8	-.2	-3.3
36.....	12	86.0	4.7	4.7	0	0
47.....	13	86.0	4.7	4.9	+.2	+4.3
Experiment 235:						
Steer 259.....	2	86.0	4.5	^b 4.0	-.5	-11.1
260.....	2	85.5	6.4	^b 6.0	-.4	-6.2
259.....	3	86.0	6.9	^b 6.5	-.4	-5.8
260.....	3	86.0	8.9	^b 8.4	-.5	-5.6
Experiment 220:						
Steer K.....	1	53.0	6.1	6.4	+.3	+4.9
K.....	3	53.0	5.9	5.6	-.3	-5.1
K.....	4	53.0	9.4	8.9	-.5	-5.3
Experiment 217:						
Steer J.....	1	53.0	6.6	6.0	-.6	-9.1
J.....	2	53.0	17.8	16.7	-1.1	-6.2
J.....	4	53.0	9.6	9.5	-.1	-1.0
Experiment 236:						
Steer J.....	1	53.0	13.3	12.6	-.7	-5.3
J.....	2	53.0	5.8	5.5	-.3	-5.2
J.....	3	53.0	7.7	6.7	-.1	-13.0
J.....	4	53.0	4.4	3.9	-.5	-11.4
J.....	5	53.0	9.6	8.9	-.7	-7.3
J.....	6	53.0	7.5	6.9	-.6	-8.0
J.....	7	53.0	4.8	4.3	-.5	-10.4
Experiment 212:						
Steer H.....	3	53.0	5.8	5.0	-.8	-13.8
H.....	4	53.0	5.4	5.1	-.3	-5.6
Experiment 211:						
Steer D.....	1	53.0	6.2	6.2	0	0
D.....	2	53.0	4.8	4.8	0	0
D.....	5	53.0	2.5	3.2	+.7	+28.0
G.....	2	53.0	4.4	3.9	-.5	-11.4
G.....	3	53.0	10.7	10.2	-.5	-4.7
G.....	4	53.0	5.0	4.7	-.3	-6.0
G.....	5	53.0	3.0	3.1	+.1	+3.3
Experiment 221 D:						
Cow 886.....	2	53.0	9.2	8.8	-.4	-4.3
886.....	3	53.0	5.0	4.8	-.2	-4.0
885.....	3	53.0	6.2	6.3	+.1	+1.6
Experiment 221 E:						
Cow 886.....	2	53.0	7.2	6.1	-1.1	-15.3
874.....	1	53.0	8.5	8.0	-.5	-5.9
874.....	2	53.0	7.7	7.2	-.5	-6.5
885.....	2	53.0	6.7	5.9	-.8	-11.9
885 ^a	3	53.0	2.0	^b 2.4	+.4	+20.0
Experiment 221 F:						
Cow 886.....	2	53.0	8.0	7.9	-.1	-1.2
874.....	1	53.0	8.4	^b 7.8	-.6	-7.1
874.....	2	53.0	4.6	^b 4.4	-.2	-4.3
874 ^a	3	53.0	2.2	^b 2.5	+.3	+13.6
887.....	1	53.0	6.2	^b 7.0	+.8	+12.9
887.....	2	53.0	4.0	^b 4.5	+.5	+12.5
887 ^a	3	53.0	2.3	^b 2.0	-.3	-13.0
Experiment 221 G:						
Cow 887.....	1	53.0	7.9	7.6	-.3	-3.8
887.....	2	53.0	3.7	3.8	+.1	+2.7
887.....	3	53.0	5.7	5.4	-.3	-5.3
887.....	4	53.0	8.5	8.1	-.4	-4.7

^a Fasting.^b Oxygen consumption was determined.

COMPUTATION OF THE INSENSIBLE LOSS OF BODY WEIGHT FROM THE RESPIRATORY PRODUCTS

In all the calorimetric experiments the water vapor, the carbon dioxide, and the methane production of the animals were quantitatively accounted for. Determinations of the oxygen consumption, however, were made in only 12 of the 77 periods. In these 12 periods it was possible to compute the insensible loss by subtracting the weight of the oxygen consumed from the sum of the weights of the water vapor, carbon dioxide, and methane produced. The values so computed are indicated in Table 1 by footnote reference *b*. This computation, obviously, could not be applied to the remaining 65 periods in which direct determinations of the oxygen consumption were lacking. It was possible, however, in these experiments, to make use of a modification of this method for computing the insensible loss by taking into consideration the gains or losses of body tissue and the extent as well as the source of oxygen used.

It is clear that the O_2 of the CO_2 does not correctly represent atmospheric oxygen consumed, because some of this O_2 might be derived from carbohydrates (as in the synthesis of fat), while, on the other hand, some of the atmospheric O_2 consumed might go to form H_2O (as in the oxidation of fat). However, the amounts of nonatmospheric oxygen excreted in CO_2 and the amount of atmospheric O_2 appearing as H_2O can be computed from the observed gains and losses of body tissue.

For the purpose of this computation it seemed permissible to neglect the katabolism of protein and to consider only the non-nitrogenous material oxidized. If this consisted only of carbohydrates—the respiratory quotient, therefore, being 1.0—the O_2 of the CO_2 could be considered as accurately representing the atmospheric oxygen consumed; but if some of the material oxidized consisted of fat, all the oxygen contained in the carbon dioxide as well as some of the oxygen of the water eliminated would have been derived from the air. On the other hand, if a formation of fat from carbohydrates took place, some of the oxygen of the carbon dioxide would have been derived from the carbohydrates consumed. On the basis of the average composition of animal fat, viz, C=76.5 per cent, H=12.0 per cent, O=11.5 per cent, the following equivalents were computed:

<i>Oxidation of 100 gm. of fat</i>		Gm.
O_2 required for oxidation of H_2		96.0
O_2 contained in fat.....		11.5
O_2 derived from the atmosphere.....		84.5

<i>Formation of 100 gm. of fat</i>		Gm.
$C_6H_{12}O_6$ required to furnish C.....		191.25
O_2 in 191.25 gm. of $C_6H_{12}O_6$		102.0
O_2 contained in 100 gm. of fat.....		11.5
O_2 of carbohydrate excreted in CO_2		90.5

In accord with the foregoing values, if to the carbon of the carbon dioxide produced is added the gain of fat multiplied by 0.905, or if from the carbon of the carbon dioxide is subtracted the loss of fat

multiplied by 0.845, the result represents the difference in weight between the CO_2 produced and the oxygen consumed. By adding to the latter result the weights of the water vapor and of the methane produced, the total insensible loss is obtained. This procedure was employed in the derivation of 65 of the computed values of insensible loss as recorded in Table 1.

COMPARISON OF THE DIRECTLY OBSERVED INSENSIBLE LOSS IN BODY WEIGHT WITH THAT COMPUTED FROM THE RESPIRATORY PRODUCTS

An examination of the data in Table 1 reveals the fact that the insensible loss as computed from the respiratory products agrees fairly well with the observed loss in body weight. The agreement between the computed and the observed values is just as close in the cases where the oxygen consumption was directly determined as in the cases where no such determinations of oxygen were made. This agreement indicates that there are, at the most, no gross errors in the values for insensible loss obtained by either of the methods used. This may also signify that it is possible to compute the O_2 consumption with a fair degree of accuracy when the CO_2 production and the gain or loss of fat are known. In the case of ruminants, however, it is quite conceivable that some of the oxygen of the carbon dioxide produced is derived from carbohydrates by process of fermentation. There is, however, no satisfactory basis available for the estimation of this quota.

It will be observed that the insensible loss in these experiments varies considerably in magnitude, the lowest observed value being 2.0 kgm. and the highest 17.8 kgm. per day. The computed values agree fairly well with the observed, being slightly larger in some cases and smaller in others. The lowest computed value is 2.0 kgm. and the highest is 16.7 kgm. per day. These large variations, as is shown later, appear to be due chiefly to differences in the plane of nutrition and in the size of the animals.

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE INSENSIBLE LOSS OF BODY WEIGHT

In addition to the determinations of the insensible loss during the calorimeter periods it was found possible to determine such values, though with somewhat less accuracy, in 71 associated digestion periods, the animals being confined in stalls, in a barn, under less rigidly controlled conditions as to temperature and ventilation than in the calorimeter. A slight element of error in these values resulted from the fact that in some cases the excreta were collected at 6 a. m., while the animal was not weighed until about 8 a. m. Also, the animal was fed at 6 a. m., and no record was kept of any feed which might have been left in the manger at the time the animal was weighed. In computing the daily insensible loss it was necessary to assume that both the feed box and the boxes for collecting the excreta were empty at the time the animal was weighed. It would seem that such an assumption might cause considerable error in the daily determination of the insensible loss. However, in the determination of the

average daily insensible loss for an experimental period it is not necessary to consider all the daily weighings of the animal or the time of such weighings; in fact, it is necessary to consider only the initial and final live weights. The digestion periods varied in length from 5 to 14 days. Hence, any error that might have arisen from the situation discussed could be, at the most, but a small fraction of the total insensible loss.

Of the 71 digestion experiments mentioned, 8 were with dry cows, 8 with lactating cows, and 55 with steers. The number of individual animals used in all these experiments was 16, of which 4 were cows, and 12 were steers.

In 24 of these digestion periods a continuous record of the temperature of the barn was obtained. Since the animals received rations of the same quantities and character during the digestion periods outside the calorimeter as during the associated periods in the calorimeter, it was possible to compare the insensible loss as determined under these different environmental conditions. Such a comparison is made in Table 2.

The average temperature in the calorimeter was higher than the average temperature in the digestion stall in all but one of the periods for which continuous records of temperature in the stall were available. It will be observed that the differences in insensible loss were not always in the same direction as the differences in temperature. In the one case (experiment 240, steer 60, period 9) in which the temperature in the calorimeter was 2.2° C. lower than the temperature in the stall, the insensible loss in the calorimeter was considerably less than the insensible loss in the stall. In six cases the insensible loss in the calorimeter was less than the corresponding insensible loss in the stall, although the temperature in the calorimeter was the higher. In 16 cases the relatively higher temperature in the calorimeter was attended by a higher insensible loss.

All the differences in insensible loss recorded in Table 2 may be viewed as representing the aggregate effect of the differences between the environmental conditions in the calorimeter and in the digestion stall. The temperature of the air in the calorimeter was always well controlled, and, as shown in the table, was with one exception appreciably higher than the recorded average temperatures in the digestion stalls in the barn. In those periods for which stall temperatures are missing, the average temperatures in the stall may be assumed to have been in most cases lower than those in the calorimeter, in view of the high temperatures maintained in the latter. The calorimeter temperatures given in the table are averages of readings taken at half-hour intervals, while the temperatures of the stall are averages of readings taken at 3-hour intervals. The temperatures of the calorimeter were quite constant, while those of the stall varied considerably during the experimental period. No observations were made on the humidity either in the stall or in the calorimeter, but in all probability differences in this respect existed. Air was admitted to the calorimeter at a definite rate, and it was agitated by means of an electric fan. The rations, however, were the same in both the stall and the calorimeter.

TABLE 2.—Influence of environmental conditions and of plane of nutrition on the insensible loss in body weight of cattle

Experiment and animal No.	Period No.	Average live weight of animal	Dry matter of feed consumed per day	Results obtained in digestion stall			Results obtained in calorimeter			Difference in temperature (temperature in calorimeter minus temperature in stall)		Difference in insensible loss	
				Number of days averaged	Average temperature of air	Average insensible loss per day	Rate of ventilation per minute	Average temperature of air	Average insensible loss per day			Insensible loss in calorimeter minus insensible loss in stall	In per cent of insensible loss in calorimeter
Experiment 240:		Kgm.	Kgm.		° C.	Kgm.	Liters	° C.	Kgm.	° C.		Kgm.	Per cent
Steer 60.....	1	311	2.8	14	-----	3.5	443	17.7	3.3	-----	-----	-0.2	-6.1
57.....	2	360	3.1	12	-----	3.9	446	17.6	4.5	-----	-----	+6	+13.3
3.....	3	333	4.2	10	-----	4.2	446	17.6	4.9	-----	-----	+7	+14.3
57.....	4	384	4.6	13	14.9	7.0	446	17.5	6.3	+2.6	-----	-7	-11.1
60.....	5	358	5.7	12	14.7	9.4	440	17.6	9.5	+2.9	-----	+1	+1.1
57.....	6	408	6.2	12	15.3	12.7	444	17.5	10.6	+2.2	-----	-2.1	-19.8
57.....	8	444	8.1	14	14.8	14.5	598	15.9	12.3	+1.1	-----	-2.2	-17.9
60.....	9	427	9.5	13	13.7	16.5	614	11.5	10.6	-2.2	-----	-5.9	-55.7
57.....	10	398	1.7	13	14.1	4.3	444	17.5	4.0	+3.4	-----	-3	-7.5
60.....	11	381	1.7	12	12.6	4.4	445	17.3	3.5	+4.7	-----	-2.9	-25.7
57.....	12	456	5.2	12	13.2	4.2	441	17.3	6.4	+4.1	-----	+2	+34.4
60.....	13	412	5.0	14	13.7	5.5	441	17.2	6.1	+3.5	-----	+6	+9.8
Experiment 221 D:													
Cow 886.....	2	424	6.1	8	8.6	6.4	675	17.6	9.2	+9.0	-----	+2.8	+30.4
886.....	3	420	3.8	8	12.2	4.0	670	17.5	5.0	+5.3	-----	+1.0	+20.0
885.....	3	426	3.9	8	12.2	4.6	677	17.6	6.2	+5.4	-----	+1.6	+25.8
Experiment 221 E:													
Cow 886.....	2	401	7.0	8	13.8	6.4	575	17.7	7.2	+3.9	-----	+8	+11.1
874.....	1	381	6.9	8	13.3	6.4	673	18.0	8.5	+4.7	-----	+2.1	+24.7
874.....	2	391	6.6	8	14.5	7.7	607	18.2	7.7	+3.7	-----	0	0
885.....	2	434	3.4	7	-----	4.3	579	17.9	6.7	-----	-----	+2.4	+35.8
Experiment 221 F:													
Cow 886.....	2	412	8.1	8	17.1	9.1	489	18.1	8.0	+1.0	-----	-1.1	-13.8
874.....	1	429	6.0	8	11.7	6.3	497	17.4	8.4	+5.7	-----	+2.1	+25.0
874.....	2	416	4.0	8	9.0	4.1	498	17.8	4.6	+8.8	-----	+5	+10.9
887.....	1	335	5.4	8	8.7	4.5	488	17.8	6.2	+9.1	-----	+1.7	+27.4
887.....	2	320	3.6	7	11.0	3.3	498	17.7	4.0	+6.7	-----	+7	+17.5
Experiment 220:													
Steer K.....	1	514	6.0	6	-----	7.0	652	17.8	6.1	-----	-----	-9	-14.8
K.....	3	490	3.9	5	-----	7.3	641	17.1	5.9	-----	-----	-1.4	-23.7
K.....	4	514	6.6	5	-----	8.9	652	18.3	9.4	-----	-----	+5	+5.8
Experiment 217:													
Steer J.....	1	490	4.5	6	-----	6.5	647	18.3	6.6	-----	-----	+1	+1.5
J.....	2	536	9.1	6	-----	18.8	644	18.4	17.8	-----	-----	-1.0	-5.6
J.....	4	642	5.2	5	-----	9.5	649	18.1	9.6	-----	-----	+1	+1.0
Experiment 218:													
Steer J.....	1	389	8.8	6	-----	12.3	650	17.8	13.3	-----	-----	+1.0	+7.5
J.....	2	367	3.8	5	-----	4.3	654	17.7	5.8	-----	-----	+1.5	+25.9
J.....	3	387	5.3	6	-----	7.5	654	17.7	7.7	-----	-----	+2	+2.6
J.....	5	404	7.9	6	-----	7.9	653	17.7	9.6	-----	-----	+1.7	+17.7
J.....	7	377	3.5	6	-----	4.4	652	17.7	4.8	-----	-----	+4	+8.3
Experiment 212:													
Steer H.....	3	354	5.3	6	-----	6.2	652	17.9	5.8	-----	-----	-4	-6.9
H.....	4	349	5.4	6	-----	5.6	657	17.8	5.4	-----	-----	-2	-3.7
Experiment 211:													
Steer D.....	1	460	6.2	6	-----	7.4	679	17.9	6.2	-----	-----	-1.2	-19.4
D.....	2	432	5.5	5	-----	4.9	650	17.9	4.8	-----	-----	-1	-2.1
D.....	3	470	7.9	6	-----	10.6	644	17.9	12.8	-----	-----	+2.2	+17.2
D.....	5	428	1.8	6	-----	4.5	672	17.8	2.5	-----	-----	+2	+30.0
G.....	2	358	2.3	6	-----	3.7	654	17.9	4.4	-----	-----	-7	-15.4
G.....	3	398	7.0	6	-----	12.7	637	17.9	10.7	-----	-----	-2.0	-18.7
G.....	4	387	3.1	6	-----	6.0	663	17.8	5.0	-----	-----	-1.0	-20.0
G.....	5	364	1.8	6	-----	3.5	666	17.7	3.0	-----	-----	-5	-16.7
Experiment 221 G:													
Cow 887.....	1	383	8.0	9	12.4	7.0	492	18.0	7.9	+5.6	-----	+9	+11.4
887.....	2	346	3.8	8	10.9	2.3	491	17.6	3.7	+6.7	-----	+1.4	+37.8
887.....	3	345	5.9	8	10.8	4.4	493	17.8	5.7	+7.0	-----	+1.3	+22.8
887.....	4	358	8.0	8	6.8	5.5	492	17.9	8.5	+11.1	-----	+3.0	+35.3
Experiment 238:													
Steer 47.....	1	486	7.4	14	-----	9.9	629	8.9	9.4	-----	-----	-5	-5.3
36.....	2	483	7.0	14	-----	9.8	621	9.7	9.4	-----	-----	-4	-4.3
36.....	3	495	5.6	14	-----	7.8	613	14.4	8.6	-----	-----	+8	+9.3
36.....	4	490	5.4	14	-----	7.0	617	14.4	7.8	-----	-----	+8	+10.3
47.....	5	475	1.9	8	-----	4.0	453	16.7	4.9	-----	-----	+9	+18.4
36.....	6	471	1.9	9	-----	4.0	448	16.7	5.0	-----	-----	+1.0	+20.0
47.....	7	485	3.8	14	-----	4.5	448	16.7	6.0	-----	-----	+1.5	+25.0
36.....	8	481	3.8	14	-----	4.7	450	16.7	5.8	-----	-----	+1.1	+19.0
36.....	10	500	5.8	14	-----	5.1	608	16.9	6.7	-----	-----	+1.6	+23.9

TABLE 2.—*Influence of environmental conditions and of plane of nutrition on the insensible loss in body weight of cattle—Continued*

Experiment and animal No.	Period No.	Average live weight of animal	Dry matter of feed consumed per day	Results obtained in digestion stall			Results obtained in calorimeter			Difference in insensible loss		
				Number of days averaged	Average temperature of air	Average insensible loss per day	Rate of ventilation per minute	Average temperature of air	Average insensible loss per day	Difference in temperature (temperature in calorimeter minus temperature in stall)	Insensible loss in calorimeter minus insensible loss in stall	In per cent of insensible loss in calorimeter
Experiment 237:		Kgm.	Kgm.		° C.	Kgm.	Liters	° C.	Kgm.	° C.	Kgm.	Per cent
Steer 47.....	2	360	4.0	11	6.7	447	17.5	5.1	-----	-1.6	-81.3	
254.....	3	345	2.0	9	3.9	445	17.6	4.0	-----	-1.1	+2.5	
47.....	4	362	3.4	14	6.4	444	17.5	5.9	-----	-1.5	-8.5	
254.....	5	347	3.3	12	4.4	451	17.7	6.2	-----	+1.8	+20.0	
47.....	6	357	4.0	8	5.3	442	17.5	6.5	-----	+1.2	+18.5	
36.....	10	330	4.0	10	5.9	445	17.6	5.2	-----	-1.7	-13.5	
47.....	11	355	4.4	7	6.8	435	17.6	6.0	-----	-1.2	-13.3	
36.....	12	319	3.5	14	5.1	443	17.7	4.7	-----	-1.4	-8.5	
47.....	13	347	3.8	14	3.3	443	17.6	4.7	-----	+1.4	+29.8	
Experiment 235:												
Steer 259.....	2	351	3.5	14	3.9	446	17.7	4.5	-----	+1.6	+13.3	
260.....	3	369	3.5	14	5.5	441	17.7	6.4	-----	+1.9	+14.1	
259.....	3	379	5.7	14	6.5	445	17.7	6.9	-----	+1.4	+5.8	
260.....	3	398	5.7	14	8.6	443	17.7	8.9	-----	+1.3	+3.4	

Of the 71 comparisons presented in Table 2, 44 showed a greater insensible loss in the calorimeter than in the digestion stall and 26 showed a greater loss in the stall than in the calorimeter.

The above observations indicate that the temperature of the environment tends to exert a definite influence on the insensible loss, but that this influence is not very pronounced, or is modified by other conditions, such as the movement of the air and the relative humidity.

INFLUENCE OF THE COAT OF HAIR ON THE INSENSIBLE LOSS OF BODY WEIGHT

In connection with a consideration of the possible influences of the environmental conditions on the insensible loss it seems to be relevant to consider the coat of hair of the animal. No detailed study of the influence of this factor has been made, but some information can be gained by referring to experiment 235 in Table 2.

In this experiment steers 259 and 260, which were of nearly the same size, received identical rations in the corresponding periods. In period 2 each of the two steers received a maintenance ration, and in period 3 each received a supermaintenance ration. Steer 259 was shorn, while steer 260 possessed his full winter coat. In both periods 2 and 3 the insensible loss of the steer having his full coat of hair was considerably greater than that of the steer which had been shorn. In period 2 the insensible loss of steer 260, in the barn, exceeded the corresponding loss of steer 259 by 1.6 kgm.; and in period 3 this excess was 2.1 kgm. In the calorimeter, under identical conditions as to temperature and ventilation, the insensible loss of steer 260 exceeded the loss of No. 259 by 1.9 kgm. in period 2 and by 2.0 kgm. in period 3. In this experiment, at least, the influence of the coat of hair appeared

to be considerably greater than the aggregate effect of the differences in environmental conditions in the barn as compared with those in the calorimeter.

INFLUENCE OF PLANE OF NUTRITION ON THE INSENSIBLE LOSS OF BODY WEIGHT

The data in Table 2 afford excellent means for studying the effect of the plane of nutrition on the insensible loss. Since the environmental conditions were nearly uniform in the calorimeter, fairly correct information regarding the influence of the nutritive plane on the insensible loss may be had by comparing the amounts of feed received by the individual animals with the values for insensible loss obtained in the calorimeter. In making such comparisons it is necessary also to consider the live weights of the animals.

It will be observed that in general, the insensible loss is closely related to the amounts of feed consumed. In nearly every experiment the lowest amounts of feed consumed by the individual animals were accompanied by the lowest values for insensible loss, and the highest amounts of feed were accompanied by the highest values for insensible loss.

The size of the animals also affects the magnitude of these values. This can best be seen by comparing the values for insensible loss of individuals receiving identical amounts of feed but varying considerably in size. Thus, in experiment 216, period 3, the insensible loss of body weight of steer J receiving 5.3 kgm. of feed and weighing 387 kgm. was 7.7 kgm. in the calorimeter as compared with 9.6 kgm. for the insensible loss of the same steer in experiment 217, period 4, on practically the same amount of feed (5.2 kgm.) but weighing 642 kgm. Similarly, it is found that the insensible loss in the calorimeter of steers 36 and 47 was greater in experiment 238, periods 7 and 8, than the values for the same steers on practically the same amounts of feed in experiment 237, periods 12 and 13, the steers being considerably heavier in experiment 238 than in experiment 237.

It is apparent, therefore, that the large variations noted in the values for insensible loss in the different periods both in the calorimeter and in the digestion stall are mainly due to differences in the plane of nutrition and in the size of the animals, the feed consumption showing the most pronounced effects.

The quantitative relation between the plane of nutrition and the insensible loss, and the value of the insensible loss as a basis for computing the heat production, are considered in a subsequent paper.⁷

SUMMARY

In this paper are presented determinations of the average daily insensible loss in body weight (excess of gaseous outgo over gaseous income) of cattle and a discussion of some factors which affect this value.

Data from 77 respiration calorimetric experiments, varying in length from 53 to 111 hours, and from 71 digestion trials of 5 to 14 days' duration, were used as the basis for the computation of the insensible

⁷Kriss, M. Op. cit

loss of steers and cows, at planes of nutrition varying from fast to about three times the maintenance requirement.

The insensible loss in body weight was computed in all experiments by a procedure based on the live weights of the animals, the weights of the feed and water consumed, and the weights of the excreta voided; and, in addition, in some experiments, the results thus obtained were checked by a computation based on the respiratory products as measured in the calorimeter. There was good agreement between the values for the insensible loss as obtained by the two methods.

The insensible losses in the calorimetric experiments varied considerably in magnitude. The lowest observed value (based on live weights) was 2.0 kgm. per day, and the highest 17.8 kgm. per day. The lowest computed value (based on the respiratory products) was 2.0 kgm. per day, and the highest 16.7 kgm. per day. These large variations are related to the differences in planes of nutrition and size of the animals.

A comparison of the data for the average daily insensible loss obtained in the digestion stall with those obtained in the calorimeter indicates that the temperature of the environment exerts a definite influence on the insensible loss, but that this influence is either not very pronounced or is modified by other conditions of the environment, such as the movement of the air and the relative humidity.

The data indicate that the coat of hair influences the insensible loss in a marked degree.

QUANTITATIVE RELATIONS OF THE DRY MATTER OF THE FOOD CONSUMED, THE HEAT PRODUCTION, THE GASEOUS OUTGO, AND THE INSENSIBLE LOSS IN BODY WEIGHT OF CATTLE¹

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INTRODUCTION

The methods necessary to the investigation of the principles of respiration and energy metabolism of man and animals—even the simpler methods of indirect calorimetry—are sufficiently complicated to preclude their general use in the solution of problems of a practical nature. This is as true in relation to animals as to human beings. As the facts relating to the energy metabolism of animals become more clearly established, however, the underlying principles come to light, and make possible the application of the fundamental truths to practical problems of animal metabolism.

One such problem—the ventilation requirements in barns—requires, for its study, a knowledge of the respiratory metabolism and the heat production of farm animals. Stables as a rule are not artificially heated, the heat supplied by the animals alone serving to temper the cold of winter weather. Another important function of the animal heat is to serve as a motive power in ventilation. The water vapor, which comes from the lungs and skin of the animals, adds to the humidity, while the carbon dioxide and the methane given off serve as continuous contaminations of the stable air. All these factors affect the comfort and, presumably, the productiveness of the animals.

Obviously, it is impracticable to employ a respiration apparatus directly in the study of this subject. It is important, therefore, to establish a method for the consideration of the matter by a process of computation. In 1921 Armsby and Kriss (1)³ proposed a method based on respiration calorimetric data, for computing the heat production, water vapor, and carbon dioxide production of farm animals, with approximate accuracy, from the live weights of the animals and the rations received. According to this method the heat production of cattle is obtained by adding to the computed maintenance requirement of net energy the total heat increment (energy cost of food utilization) of the ration, this quota being estimated on

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³ Reference is made by number (italic) to "Literature cited," p. 295.

the basis of average heat-increment values; while the CO_2 production is computed from the heat- CO_2 ratio of Armsby, Fries, and Braman (2).

It is unfortunate that the number of experimentally determined heat-increment values of feeding stuffs is very small, even for cattle. This fact considerably limits the usefulness of the method referred to; and a modification seems desirable, as has been recently suggested by Kelley (7).

In an effort to provide an adequate basis for the computation of the amounts of the heat production and the gaseous products of cattle, for practical uses, especially as applying to the solution of problems of ventilation of barns, the writer has made a biometric study of the measurements indicated below, all of which were derived from published respiration calorimetric experiments of this institute.

This study has been directed to the establishment of the relationship between (1) the dry matter of the feed consumed and the insensible loss; (2) the insensible loss and the heat production; (3) the dry matter of the feed consumed and the heat production; (4) the carbon dioxide and the heat production; (5) the water vapor and the heat production; (6) the dry matter of the feed consumed and the carbon dioxide production; (7) the dry matter of the feed consumed and the water vaporized; and (8) the dry matter of the feed consumed and the methane production.

DATA SUBJECTED TO STATISTICAL ANALYSIS

The data analyzed comprise two series.

FIRST SERIES

For the purpose of determining the correlation between the dry matter of the food consumed and the insensible loss, and the correlation between the insensible loss and the heat production, the results of 74 experimental periods were used, including all except the fasting periods indicated in Table 1 of the foregoing paper (8). Of these 74 experiments, 58 were with steers, 8 with dry cows, and 8 with lactating cows. The number of individual animals used as subjects in these experiments was 16, of which 4 were cows and 12 were steers. The average live weights of the animals varied from 311 to 642 kgm. The feed consumption in these experiments varied from 392 to 2,268 gm. of dry matter per 100 kgm. of live weight per day. In this series the following measurements were considered: (1) Insensible loss per 100 kgm. of live weight per day; (2) heat production per 100 kgm. of live weight per day; and (3) dry matter of feed eaten per 100 kgm. of live weight per day.

The data for insensible loss used in this analysis are those obtained under the well-controlled conditions in the respiration calorimeter. The values for heat production of this, as well as of the following series, are as directly observed—uncorrected for difference in time spent standing and lying.

SECOND SERIES

This series comprises the results of 131 experimental periods, which include 66 of the first series (excluding the experiments with lactating cows). Of the total number of experiments 123 were with steers and 8 with dry cows. The number of individual animals used as subjects

in these experiments was 24, of which 20 were steers and 4 were cows. The average live weight of the animals varied from 162 to 655 kgm. The feed consumption varied from 392 to 2,268 gm. of dry matter per 100 kgm. of live weight per day. In this series the following measurements were considered: (1) Heat production per 100 kgm. of live weight per day; (2) carbon dioxide production per 100 kgm. of live weight per day; (3) water vaporized per 100 kgm. of live weight per day; (4) dry matter of feed eaten per 100 kgm. of live weight per day; (5) dry matter of feed eaten per head per day; and (6) methane production per head per day.

METHODS AND RESULTS OF STATISTICAL ANALYSIS

The statistical methods used are those of the standard text books on biometry, but particularly the methods and formulas discussed and used by Harris and Benedict in their *Biometric Study of Basal Metabolism in Man* (6).

The main results sought in this analysis were coefficients of correlation between the various measurements considered, and regression equations. The computations of the statistical constants, such as the means, the standard deviations and the coefficients of variation, are incidental to the main purpose of this analysis.

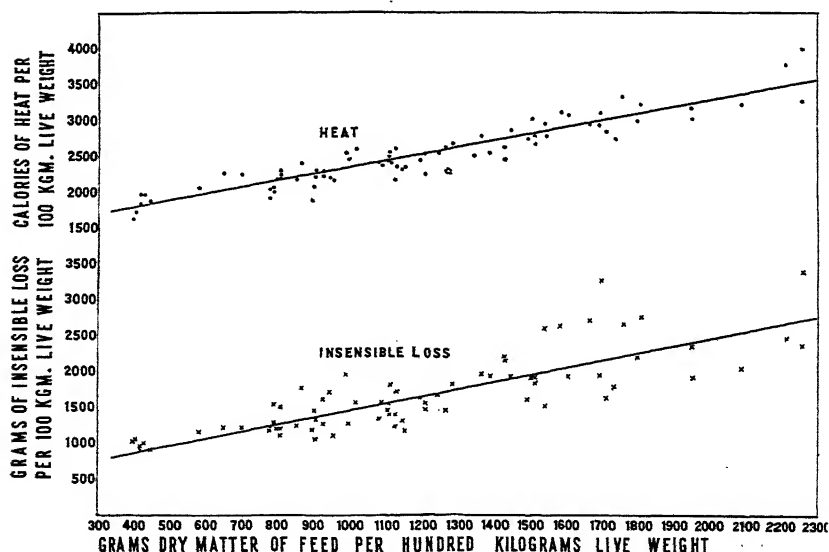


FIGURE 1.—Relationships (1) between dry matter of feed consumed and heat production and (2) between dry matter of feed consumed and insensible loss in body weight of cattle

All the data analyzed are represented graphically in Figures 1 to 5. The data of the first series are shown in Figures 1 and 2. In Figure 1 the insensible loss and the heat production are plotted against the dry matter of feed consumed, or, more specifically, the grams of dry matter of feed per 100 kgm. of live weight were used as abscissas, and the grams of insensible loss and Calories of heat per 100 kgm. of live

weight were used as ordinates. This, therefore, exhibits diagrammatically the relation between the dry matter of feed consumed and the insensible loss, and the relation between the dry matter of feed consumed and the heat production of the animal. In Figure 2 the heat production is plotted against the insensible loss, and the relationship between these two measurements is shown.

The statistical analysis of the data represented by Figures 1 and 2 reveals that there is a fairly close correlation between the dry matter of the feed consumed and the insensible loss and between insensible loss and heat production, but that there exists a considerably closer correlation between the dry matter of the feed consumed and the heat production than between the insensible loss and the heat production. The following correlation coefficients were obtained: Between dry matter of feed and insensible loss, 0.818 ± 0.026 ; between insensible loss and heat production, 0.847 ± 0.022 ; and between dry matter of feed and heat production, 0.936 ± 0.010 .

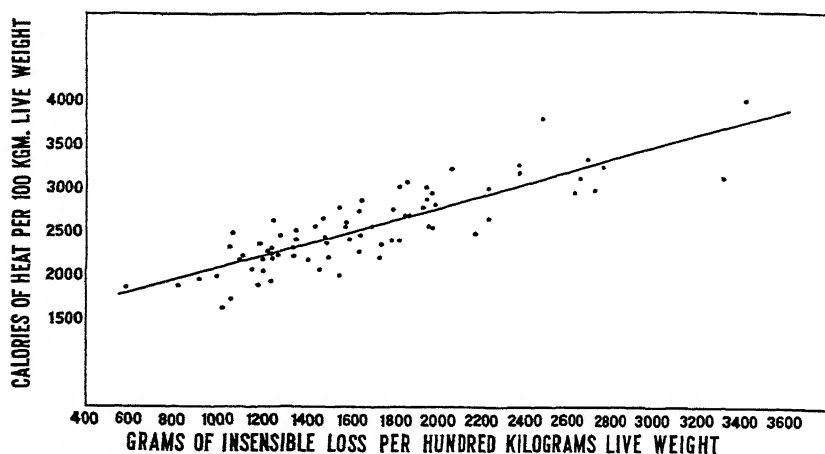


FIGURE 2.—Relationship between the insensible loss in body weight and the heat production of cattle

Figures 3, 4, and 5 represent data of the second series referred to above.

In Figure 3 the carbon dioxide production, water vapor, and heat production are plotted against the dry matter of the feed consumed. These data gave upon analysis the following coefficients of correlation: Between dry matter of feed and heat production, 0.883 ± 0.013 ; between dry matter of feed and CO_2 production, 0.938 ± 0.007 ; and between dry matter of feed and water vapor, 0.762 ± 0.025 .

In the first series the coefficient of correlation between dry matter of feed consumed and heat production is somewhat higher than that in the second series. The latter was based on a considerably larger number (131) of measurements than the former (74) and is therefore considered as more significant.

There is a very close correlation between the dry matter of the feed consumed and carbon dioxide production, as is indicated by the high coefficient (0.938). The lowest coefficient of correlation was found between the dry matter of feed and the water vapor. This could be anticipated.

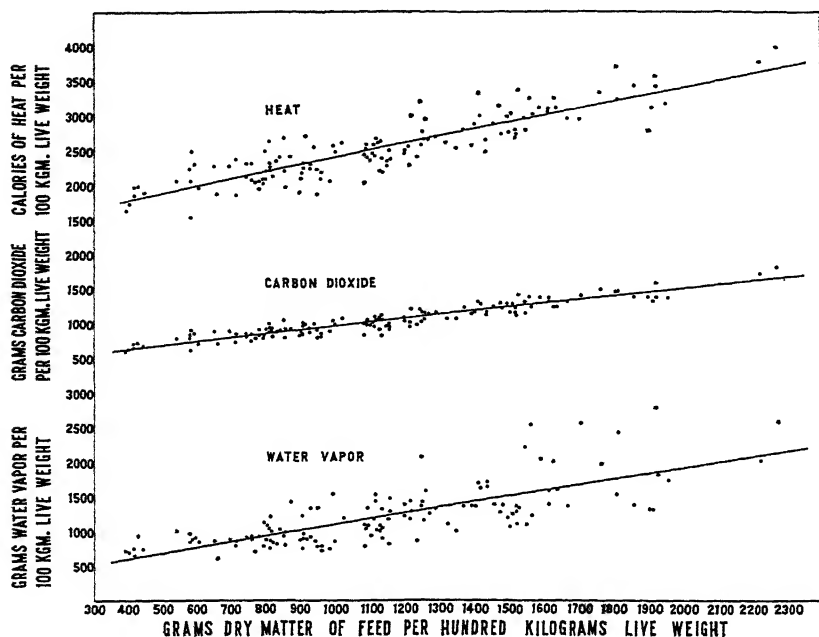


FIGURE 3.—Relationships (1) between dry matter of feed consumed and heat production, (2) between dry matter of feed consumed and carbon dioxide production, and (3) between dry matter of feed consumed and water vapor production of cattle

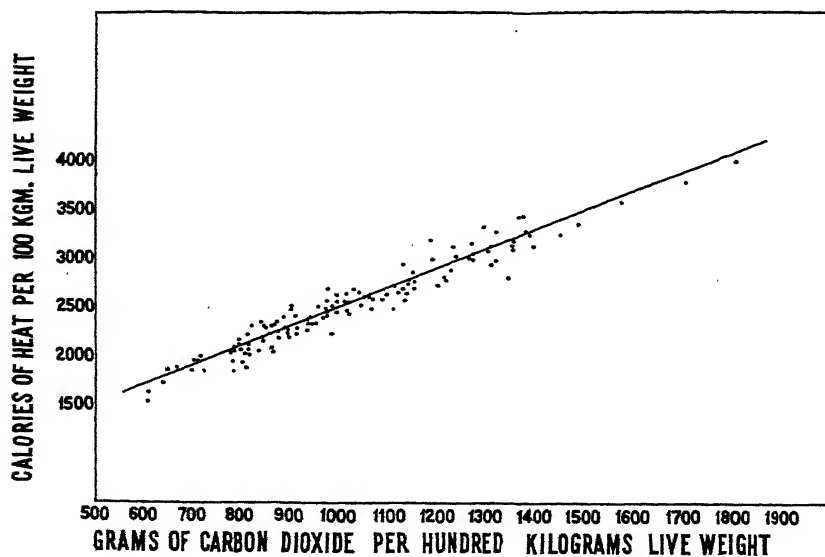


FIGURE 4.—Relationship between carbon-dioxide production and heat production of cattle

The correlation between the carbon dioxide production and the heat production is indicated graphically in Figure 4, in which calories of heat were plotted against grams of carbon dioxide. These data gave upon analysis the very high coefficient of correlation of 0.967 ± 0.004 .

In all of the foregoing statistical considerations the data were computed to a uniform basis of live weight, namely, per 100 kgm. This was done to eliminate, or at least to minimize, the effect of the factor of live weight, since the measurements considered are functions of both the size of the animal and the rations consumed.

In Figure 5 the methane production per head was plotted against the dry matter of feed consumed per head. Since methane fermentation is a function of the feed, and not of the size of the animal, it was not deemed necessary in this case to take into account the live weights

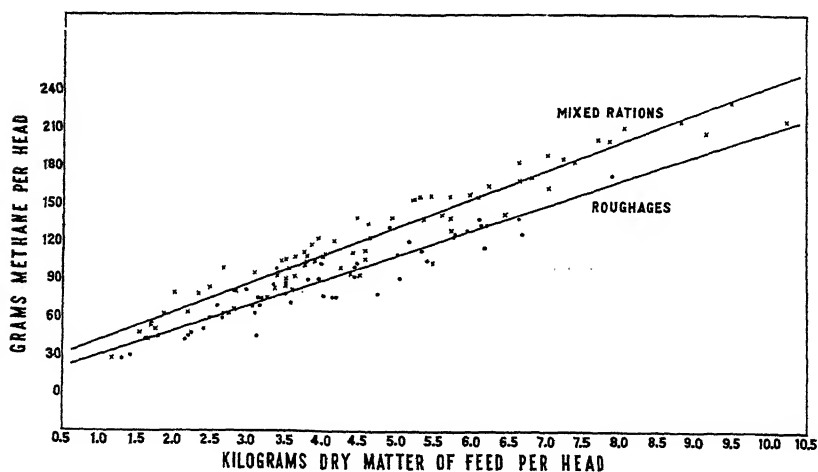


FIGURE 5.—Relationship between dry matter of feed consumed and methane production of cattle

of the animals. The coefficient of correlation between the methane production and the dry matter of feed, for all the experiments, was found to be 0.937 ± 0.007 .

Of the 131 experiments of the second series 54 were on roughage and 77 were on rations consisting of roughage plus grain. In consideration of (1) the size of these two groups, (2) the greater content of digestible carbohydrates in rations containing grain, and (3) the known fact that methane fermentation is directly affected by the digestible carbohydrates of the rations, the data for dry matter and methane of the above two groups were analyzed separately, giving 0.942 ± 0.010 as the coefficient of correlation for the roughage rations and 0.963 ± 0.006 as the coefficient for the mixed rations.

The results of the statistical analysis of all the data under consideration are summarized in Tables 1 and 2. Table 1 presents the statistical constants, while the results of the final calculations, that is, the correlation coefficients and the prediction equations are presented in Table 2.

TABLE 1.—Statistical constants for the daily measurements in the respiration calorimetric experiments

Kind of measurement	Number of experiments	Range	Mean	Standard deviation	Coefficient of variation
Grams dry matter of feed per 100 kgm. live weight.....	74	392-2,268	1,208.608± 36.059	459.862± 25.476	38.05±2.40
Grams insensible loss per 100 kgm. live weight.....	74	584-3,419	1,668.554± 43.644	556.603± 30.836	33.36±2.05
Calories of heat per 100 kgm. live weight.....	74	1,633-3,995	2,538.446± 35.519	482.981± 25.095	17.84±1.02
Grams dry matter of feed per 100 kgm. live weight.....	131	392-2,268	1,134.237± 23.825	404.500± 16.868	35.66±1.67
Calories of heat per 100 kgm. live weight.....	131	1,531-3,995	2,528.771± 27.672	469.814± 19.591	17.45± .75
Grams carbon dioxide per 100 kgm. live weight.....	131	609-1,811	1,028.878± 13.626	231.346± 9.647	22.14± .97
Grams water vapor per 100 kgm. live weight.....	131	572-2,803	1,200.221± 25.498	432.897± 18.052	36.07±1.69
Grams dry matter of feed per head.....	131	1,151-1,022	4,308.557±109.739	1,863.135± 77.618	41.08±1.98
Grams methane per head.....	131	27- 230	106.611± 2.642	44.859± 1.871	42.04±2.04
Grams dry matter of feed per head.....	^a 54	1,300-7,893	3,946.241±140.662	1,532.381± 99.452	38.83±2.88
Grams methane per head.....	^a 54	27- 173	86.833± 2.949	32.129± 2.085	37.00±2.71
Grams dry matter of feed per head.....	^b 77	1,151-1,022	4,562.649±155.685	2,025.408±110.182	44.39±2.85
Grams methane per head.....	^b 77	27- 230	120.481± 3.632	47.255± 2.571	39.22±2.44

^a Experiments on roughages only.^b Experiments on mixed rations.

TABLE 2.—Correlation coefficients of various pairs of measurements in the respiration calorimetric experiments, and prediction equations

x-variable *	y-variable *	Number of experiments	Coefficient of correlation	Prediction equation
Dry matter of feed.....	Insensible loss.....	74	0.618±0.026	y=0.9900x+ 472
Insensible loss.....	Heat production.....	74	.847± .022	y= .6892x+1,389
Dry matter of feed.....	do.....	74	.936± .010	y= .9224x+1,424
Do.....	do.....	131	.883± .013	y=1.0250x+1,366
Carbon dioxide.....	do.....	131	.967± .004	y=1.9640x+ 508
Water vapor.....	do.....	131	.800± .021	
Dry matter of feed.....	Carbon dioxide.....	131	.938± .007	y= .5362x+ 421
Do.....	Water vapor.....	131	.762± .025	y= .8151x+ 276
Do.....	Methane.....	131	.937± .007	y= .0226x+ 9
Do.....	do.....	^b 54	.942± .010	y= .0198x+ 9
Do.....	do.....	^c 77	.963± .006	y= .0225x+ 18

* All values represented are per 100 kgm. live weight, except those for methane and the dry matter to which methane is related, which are per head.

^b Experiments on roughages only.^c Experiments on mixed rations.

The results of Table 1 do not require extended comment; but in order that one may have a full appreciation of the significance of these data, especially in relation to variability, it is important to bear in mind the conditions represented by them.

In the first series, numbering 74 experiments, the data were derived from 58 experiments with steers, 8 experiments with lactating cows, and 8 experiments with dry cows. The animals varied in size from 311 to 642 kgm. live weight. The planes of nutrition varied from approximately one-half of the maintenance requirement to about three times maintenance. This extent of variation in plane of nutrition is indicated approximately by the range of variation in the

amount of dry matter of feed consumed. The rations can be characterized only very generally by the statement that in 17 experiments they consisted of roughages only, and in the remaining 57 experiments they consisted of a mixture of roughage and grain.

In the second series, comprising 131 experiments, the range of variation in feed consumption was the same as in the first series. Of the total number of experiments represented only 8 were with cows, and those were in dry condition. The remaining 123 were with steers. In some of these experiments the animals were quite young and of small size, while in others the subjects were mature. The average live weight varied from 162 to 655 kgm. In 54 experiments of this series the rations consisted of roughages only, while in 77 the rations were mixed.

In the light of the above-mentioned conditions the statistical constants presented in Table 1 are readily understandable, and are consistent.

The results exhibited in Table 2 are of theoretical as well as of practical significance.

It is of physiological interest to know how the heat production, the insensible loss, and the individual gaseous products, namely, the carbon dioxide, water vapor, and methane, are related to the feed consumption; as it is also of interest to know the degrees of interdependence of the heat production, the insensible loss, the water vapor, and the carbon dioxide. The interdependence of these measurements is indicated by the coefficients of correlation.

All the correlation coefficients shown in Table 2 are numerically high, and in comparison with their probable error are certainly to be regarded as significant. The highest correlation (0.967) is between the carbon dioxide production and the heat production. The correlation between the dry matter of feed and the insensible loss is the third lowest (0.818) of the series, that between the dry matter of the feed and water vapor being the lowest (0.762). The numerical values of these coefficients, as well as the data shown in Figures 1 and 3, indicate that the insensible loss and the water vapor—which constitutes the greater part of the insensible loss—are directly affected by the level of feeding; but the influence of other factors, such as have been discussed in the preceding paper (8), on the insensible loss, and presumably largely on the water vapor, are also apparent.

The prediction equations given in Table 2 are equations of a straight line, and represent the lines of regression shown in the several graphs previously referred to.

As regards the relation of the heat production to the dry matter of the feed, it has been demonstrated quite conclusively by Forbes, Braman, Kriss, et al. in recent investigations of this institute (4, 5) that the heat production of cattle from fast to two or three times maintenance is not accurately represented by a straight line, but is represented by a curve of a higher order. Even between the planes of half maintenance and three times maintenance the heat production of an individual animal would not be accurately represented by a straight line, according to the evidence just cited. However, from a statistical point of view, the results on various feeds and numerous individual animals having been considered together, it appears justifiable although not quite in harmony with the established facts,

to represent the relation of the heat production to the dry matter of feed, within the range here considered, by the slope of a straight line, especially inasmuch as the linearity of regression of the heat production on the dry matter is evidenced by the graphical representation of the data (figs. 1 and 3), and by the high coefficients of correlation between the heat production and the dry matter of feed shown in Table 2.

APPLICATION OF PREDICTION EQUATIONS

The application of the prediction equations to the computation of the unknown variables is extremely simple, but it might be desirable to illustrate it by means of an example. The following is an example of the computation of the heat production (y —variable) from the carbon dioxide production (x —variable).

The carbon dioxide production of a steer weighing 486 kgm. per day was 5,950 gm. The daily production of carbon dioxide per 100 kgm. live weight is, therefore, 1,224 ($5,950 \div 4.86$) gm. This value is represented by x in the equation $y = 1.964x + 508$, in which y represents heat production per 100 kgm. live weight. Substituting 1,224 for x in the equation, we find $y = 1.964$ times $1,224 + 508 = 2,912$ Cals. The daily heat production per head is, therefore, 14,152 (2,912 times 4.86) Cals.

In a similar manner the heat production of this steer may be computed from the dry matter of the feed consumed by substituting the number of grams of dry matter per 100 kgm. of live weight, which was 1,519, for x in the equation $y = 1.025x + 1,366$ and solving for y ; and then multiplying the result by 4.86. The heat production thus computed would equal 14,206 Cals.

It is evident from the above example that it is essential to know the live weights of the animals in making use of the prediction equations in which x and y represent values per 100 kgm. of live weight. The computation of methane production from the dry matter of feed does not require a consideration of the live weights, since the x and y represent values per head. The heat production is expressed in Calories, and all the other measurements must be expressed in grams.

The computation of the heat production from the carbon dioxide production, as illustrated by the above example, differs fundamentally from the heat- CO_2 method of Armsby, Fries, and Braman (2) and of Braman (3). The principal difference lies in the fact that the computation of the heat production from the equation representing the direct relationship between heat production and CO_2 production, given in Table 2, and as used in the example cited, is direct and does not require any consideration of feed consumption. According to the heat- CO_2 ratio method, however, it is necessary first to compute the ratio of heat to CO_2 from an equation representing the relationship between heat- CO_2 ratios and air-dry matter of feed; and then the heat is computed by multiplying the ratio found by the known amount of CO_2 . In view of the fact that the kind and composition of the feed must, of necessity, be disregarded in the determination of the heat- CO_2 ratio relating to the given feed, it would appear that the latter procedure makes the computation of the heat production from the CO_2 production unnecessarily complicated.

A comparison of the equations representing the relationship between the methane and the dry matter of roughage rations and mixed rations shows that the mixed rations give rise to a higher production of methane than do the roughages. This difference, amounting to 12 gm. of methane per kilogram of dry matter of feed, is indicated by the slight difference in the slope of the lines (fig. 5) represented by the equations and by the difference in the constant, and is attributable to the relatively larger amounts of digestible carbohydrates in the dry matter of the mixed rations than in the roughage rations.

An important question to consider in regard to the practical use of the data of Table 2 is the relative accuracy with which the heat production and the various gaseous products can be computed from the equations. The coefficients of correlation are a fair but not always a true index of such accuracy. For example, it would not be correct to assume that the methane production can be computed from the dry matter of feed with an accuracy of the same order as would characterize the computation of the carbon dioxide from the dry matter, on the strength of the fact merely that the coefficients of correlation are identical and are based upon the same number of experiments. The relative size of the means of the dependent variable seems to play an important rôle in this regard, in addition to the other two factors mentioned. Thus, one may expect to obtain a greater accuracy in the computation of the carbon dioxide production (which has a much greater mean numerical value than methane) from the dry matter of feed than in the computation of the methane production from the same. It would seem to be reasonable, however, to assume, on the basis of a comparison of correlation coefficients representing the same number of experiments, that the evaluation of the heat production from the carbon dioxide is more nearly accurate than is the evaluation of the same from the dry matter, and that the heat production can be computed from the dry matter of the feed with an accuracy of a higher order than that obtained in the computation of the same from the insensible loss.

A test of the relative accuracy with which the heat production can be computed by means of each of the three different prediction equations, and of the order of accuracy of the computation of the various gaseous products from the dry matter of the feed, is afforded by Table 3, in which a comparison is made of the computed values with the values which were experimentally determined, the data from several early and several recent experiments being used for this purpose.

The dry matter-heat equation used in this comparison was that based on the larger number of experiments, namely, $y = 1.0250 + 1366$. The equation used for computing the methane was $y = 0.0226x + 9$.

The average percentage differences between the computed and the directly determined values in Table 3, disregarding the signs, are: Carbon dioxide computed from dry matter of feed, 4.7 per cent; methane computed from dry matter of feed, 10.6 per cent; water vapor computed from dry matter of feed, 14.9 per cent; heat production computed from insensible loss, 7.9 per cent; heat production computed from dry matter of feed, 4.9 per cent; heat production computed from carbon dioxide, 2.9 per cent.

TABLE 3.—A comparison of directly determined values for heat production, carbon dioxide, methane, and water vapor with values computed from the prediction equations

Experiment and animal No.	Period No.	Differences between actual and computed values for—					
		Water vapor	Methane	Carbon dioxide	Heat production from—		
					Insensible-loss equation	Dry-matter equation	Carbon-dioxide equation
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Experiment 238:							
Steer 47.....	1	+18.1	-3.9	+0.9	+4.7	+5.0	+4.6
36.....	2	+6.2	+3.1	-2.5	-5.0	-5	+1.9
47.....	3	-8.7	-3.6	+3	+9.5	+7.1	+6.9
36.....	4	-12.8	-5.8	-2.1	+2.8	+2.8	+4.5
47.....	5	-17.9	-17.6	+3.6	+28.6	+8.4	+4.4
36.....	6	-15.0	-16.8	-1.0	+22.4	+2.6	+2.1
47.....	7	+1.5	-13.2	+4.3	+15.8	+12.0	+8.0
36.....	8	+2.1	-15.3	+2	+8.5	+6.0	+5.4
47.....	9	-4.3	+10.7	+15.7		+13.1	+9
36.....	10	+4.0	+12.3	+9.6	-6	+9.5	+1.8
Experiment 237:							
Steer 47.....	2	-21.6	-9.2	-6.7	-7.9	-2.2	+3.4
254.....	3	-21.8	-31.4	-8.6	+6.2	-4.8	+1.1
47.....	4	-21.2	-14.0	+1	+3.7	+1.4	+1.0
254.....	5	-31.4	-3	-3.0	+8.8	-6.2	-4.2
47.....	6	-17.1	-4.0	+7.9	+10.3	+4.7	-1.5
254.....	8	-29.0	-8.0	-6.6	-7.8	-6.4	-1.2
36.....	10	-11.0	+29.6	+7.5	-3.1	+2.3	-3.3
47.....	11	-5.2	+18.5	+7.0	-3	+3.3	-2.1
36.....	12	+1.1	-9.1	+1.7	-1.7	+2.4	+1.0
47.....	13	+6.6	-8.6	+2.2	-3.7	+2.8	+1.0
Experiment 235:							
Steer 259.....	2	+27.7	+12.4	-2.5	-7.7	-2.9	-1.1
260.....	2	-21.2	+3.8	+3.5	+16.7	+5.7	+2.6
259.....	3	+19.9	+2	-3.7	-12.6	-3.7	-3
260.....	3	-14.5	+8.0	+4.3	+10.8	+7.4	+4.0
Experiment 211:							
Steer D.....	1	+36.5	+12.2	+9.1	-7.6	+9.5	+2.3
D.....	2	+9.3	-16.7	-4.9	-3.0	-1.0	+2.5
D.....	3	+20.8	-6.7	-4	+10.1	+3.9	+4.8
D.....	4	+27.3	+1.2	+7.0		-4.7	+1.8
D.....	5	-4.5	+7.3	-1.0	-3.6	-3.5	-3.8
G.....	1	+25.8	+7.1	+1.3		-1.3	-2.0
G.....	2	-5.2	-20.9	-14.0	-2.4	-1.2	-1.1
G.....	3	-23.2	-9.8	-8.4	-2.9	+5.1	+3.0
G.....	4	-6.4	+6.9	-1.2	-3.5	-4.7	-4.2
G.....	5	-14.5	+5.4	-1.8	+3.5	-3.8	-3.5
Experiment 221 F:							
Cow 874.....	1	+3.5	-8.8	+2.7	+7.1	+9.3	+7.3
874.....	2	+35.4	-7.9	+10.6	-1.4	+7.8	-5
887.....	1	+6	-16.1	-5.2	-13.7	-1.9	+3.0
887.....	2	+11.8	-15.8	-6.6	-14.2	-3.6	+1.8
Average.....		14.9	10.6	4.7	7.9	4.9	2.9

The data of Table 3 show, therefore, in terms of percentage that the computation of the carbon dioxide from the dry matter of the feed is relatively more accurate than is the computation of the methane from the same; that the computation of the water vapor is the least accurate; that the heat production can be computed from the carbon dioxide with a close approximation to the truth; and that the dry matter of the feed is a better basis for predicting the heat production than is the insensible loss.

Obviously, if the signs of the individual differences between the actual and the computed values in Table 3 were taken into account, the average differences would be considerably smaller than those indicated above.

It should be realized, however, that the experimental data upon which the results of Table 3 are based constitute part of the data from which the prediction equations under consideration were deduced, and that the results of Table 3 do not indicate the maximum deviations from the truth possible on application of these equations to other data.

It seems safe to assume that these prediction equations would apply more nearly correctly to large groups of animals (a barnful, for example) than to single individuals, and that these equations do not provide means for the computation of the heat production and of the various gaseous products of an animal with a sufficient degree of accuracy for the most critical purposes.

SUMMARY

This paper reports numerical and graphic results of a biometric study of relations between values representing the feed consumption, the production of heat, carbon dioxide, water vapor, and methane, and the insensible loss in body weight of cattle, derived from published respiration calorimetric experiments of the Institute of Animal Nutrition of the Pennsylvania State College.

Two series of data were subjected to statistical analysis. Those of the first series were derived from 74 experimental periods (58 with steers, and 16 with cows), and included data for insensible loss, heat production, and dry matter of feed, these experiments representing planes of nutrition varying between about one-half maintenance and about three times the maintenance energy requirement. The second series comprised the results of 131 experimental periods (123 with steers and 8 with cows), including data for heat, the production of carbon dioxide, methane, and water vapor, and for the dry matter of the feed, of rations representing planes of nutrition varying as in the first series.

In the first series the insensible loss and the heat production were each correlated with the dry matter of the feed, and the insensible loss was correlated with the heat production. In the second series the heat production, the carbon dioxide, the water vapor, and the methane were each correlated with the dry matter of the feed, and the carbon dioxide and the water vapor were each correlated with the heat production. The correlation between the methane and the dry matter of the feed was also determined separately for two groups of data of the second series, one representing roughage rations, the other representing mixed rations consisting of roughage and grain. The following correlation coefficients were obtained: Between dry matter of feed and insensible loss, 0.818 ± 0.026 ; between dry matter of feed and carbon dioxide, 0.938 ± 0.007 ; between dry matter of feed (all rations) and methane, 0.937 ± 0.007 ; between dry matter of feed (roughages) and methane, 0.942 ± 0.010 ; between dry matter of feed (mixed rations) and methane, 0.963 ± 0.006 ; between dry matter of feed and water vapor, 0.762 ± 0.025 ; between dry matter of feed and heat production (74 periods), 0.936 ± 0.010 ; between dry matter of feed and heat production (131 periods), 0.883 ± 0.013 ; between carbon dioxide and heat production, 0.967 ± 0.004 ; between insensible loss and heat production, 0.847 ± 0.022 ; between water vapor and heat production, 0.800 ± 0.021 .

Regression equations were worked out from the data by means of which the heat production, the carbon dioxide, the water vapor, and the methane production can be computed, with various degrees of accuracy, from the dry matter of the feed consumed; and similar equations were worked out by means of which the heat production can be computed from either the carbon dioxide production, the insensible loss, or the dry matter of the feed.

A comparison of the computed values (by the use of the regression equations) with the values which were directly determined in a number of experiments of this institute, gave the following average, percentage differences, when the signs of the individual differences were disregarded: Carbon dioxide computed from dry matter of feed, 4.7 per cent; methane computed from dry matter of feed, 10.6 per cent; water vapor computed from dry matter of feed, 14.9 per cent; heat production computed from insensible loss, 7.9 per cent; heat production computed from dry matter of feed, 4.9 per cent; heat production computed from carbon dioxide, 2.9 per cent.

The conclusion is drawn that for practical purposes the dry matter of the feed consumed is a better basis for predicting the heat production of cattle than would be the insensible loss in body weight.

LITERATURE CITED

- (1) ARMSBY, H. P., and KRISS, M.
1921. SOME FUNDAMENTALS OF STABLE VENTILATION. *Jour. Agr. Research* 21: 343-368.
- (2) ——— FRIES, J. A., and BRAMAN, W. W.
1920. THE CARBON DIOXIDE: HEAT RATIO IN CATTLE. *Natl. Acad. Sci. Proc.* 6: 263-265.
- (3) BRAMAN, W. W.
1924. THE RATIO OF CARBON DIOXIDE TO HEAT PRODUCTION IN CATTLE. *Jour. Biol. Chem.* 60: 79-88, illus.
- (4) FORBES, E. B., BRAMAN, W. W., and KRISS, M., with the collaboration of JEFFRIES, C. D., SWIFT, R. W., FRENCH, R. B., and others.
1928. THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION. *Jour. Agr. Research* 37: 253-300, illus.
- (5) ——— BRAMAN, W. W., and KRISS, M., with the collaboration of SWIFT, R. W., FRENCH, R. B., SMYTHE, C. V., and others.
1930. FURTHER STUDIES OF THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION. *Jour. Agr. Research* 40: 37-38, illus.
- (6) HARRIS, J. A., and BENEDICT, F. G.
1919. A BIOMETRIC STUDY OF BASAL METABOLISM IN MAN. 266 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 279.)
- (7) KELLEY, M. A. R.
1924. THE RELATION OF FARM ANIMALS TO PROPER VENTILATION OF BARNs. *Agr. Engin.* 5: 155-157, illus.; [Summary] in *Amer. Soc. Anim. Prod. Proc.* 1923: 99-102.
- (8) KRISS, M.
1930. THE INSENSIBLE LOSS IN BODY WEIGHT OF CATTLE. *Jour. Agr. Research* 40: 271-281.

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PROTEIN CONTENT OF REED CANARY GRASS ON PEAT SOILS¹

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INTRODUCTION

Reed canary grass (*Phalaris arundinacea* L.), has been the subject of experiments for nearly 200 years, yet there is little agreement at present on either its nutritive value or its possible economic importance as a hay crop. From time to time during the past hundred years it has received more or less attention in this country without attaining importance as a crop, except recently and locally, and then mainly close to the north Pacific coast (15, p. 230²; 16, p. 16; 2, p. 3).

Feldt, who has recently given the grass some critical study and is working on its improvement, speaks disparagingly of it as a deceiver because in the apparently great yields the content of dry matter and digestible protein is small (7, p. 137), and on sites adapted to it he recommends *Beckmannia erucaeformis* as a better meadow grass. Weber (24), on the other hand, in his recent (1928) monograph on *Phalaris arundinacea*, states that it provides an excellent hay, rich in protein, if cut at the proper stage of development, and under favorable conditions gives yields much in excess of those of the common grasses, even as much as 9 tons per acre of cured hay from three cuttings under the most favorable conditions in northeastern Germany.

The original purpose of the present study was to compare reed canary grass with timothy, considering both yield and protein content of the hay, as a meadow crop for peat soils that are very poorly drained or are subject to overflow, cutting both grasses at the usual stage of development, that is, early bloom, but the appearance of Weber's monograph directed attention to the importance of considering both soil conditions and time of mowing.

PREVIOUS PROTEIN DETERMINATIONS

Less than 30 previous determinations of the protein content of *Phalaris arundinacea* have been found. In Table 1 these are arranged in chronological order showing the percentages in the moisture-free grass. There seems no reason to suspect that any nitrogen fertilizers had been applied in the case of any of the first 20. Some of the samples analyzed were taken from meadows of almost pure stands of reed canary grass, but most of the first 13 consisted of few wild plants.

The earliest analysis is that by Gasparin in 1848. In what he designated "normal hay," but cut before flowering, he found 1.49

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² Reference is made by number (italic) to "Literature cited," p. 319.

per cent nitrogen—equivalent to 10.95 per cent protein on a dry basis (10, p. 387). The next analysis was by Ritthausen and Scheven, who found only 7.17 per cent protein. They had collected the plants on June 12, before bloom, but instead of analyzing at once kept them in water until after bloom (13, p. 77). About the same time Hosford in Massachusetts separated some of the plants into three parts—leaves, joints, and stalks without joints or leaves—and analyzed these separately. No mention is made of the stage of development of the plants (9, p. 83). His analyses showed: Leaves, formed 41 per cent of total dry weight with 1.13 per cent N; joints, 7 per cent of total dry weight with 0.45 per cent N; and stalks without joints or leaves, 52 per cent of total dry weight with 0.08 per cent N.

TABLE 1.—Previously reported determinations of protein content of *Phalaris arundinacea* on a moisture-free basis

Reference No.	Year	Analysts	Country or State	Protein content	Time of sampling or stage of maturity	Remarks
1	1848	Gasparin (10).....	France.....	<i>Per cent</i> 10.95	Probably before bloom.	
2	1855	Ritthausen and Scheven (13).....	Germany.....	6.17	After bloom.....	
3	1856	Hosford (9, p. 83).....	Massachusetts.....	3.44	-----	
4	1859	Arendt and Knop (1).....	Germany.....	10.42	After bloom.....	Individual plants 80 to 90 inches high.
5	1876	} Storer (22).....	Massachusetts	11.06	June 13, before heading.	On part buds fully developed, 24 to 54 inches high.
6	1876			12.36	June 15, before heading.	Buds fully developed, 42 inches high.
7	1889	Hills (12).....	Vermont.....	15.09	-----	
8	1889	Cassidy and O'Brine (4).....	Colorado.....	7.78	-----	
9	1890	Wade and Patrick (20).....	Iowa.....	16.88	June 10.....	Plants 36 inches high.
10	1890	Stebler (21).....	Switzerland.....	6.90	July 26.....	
11	1893	Erwin (6).....	Utah.....	12.64	-----	
12	1893	Kellgren and Nilson (14).....	Sweden.....	10.16	First part of July.....	
13	1894	Shepard and Williams (19).....	South Dakota.....	8.20	June 20.....	
14	1908	} Rindell (17).....	Finland.....	6.93	In bloom.....	On sandy loam.
15				10.89	do.....	On reclaimed peat.
16				12.42	do.....	On light loam.
17				8.63	Ripe straw with seed removed.	Do.
18				12.40	10 days before bloom.	On humus loam.
19				7.81	In bloom.....	Do.
20				9.73	do.....	On reclaimed peat.
21				22.00	May 19, first cutting.	Fertilized on April 15 with 46 pounds per acre of N. supplied as Leunsaalpetter.
22				11.33	June 9, first cutting.	After No. 21.
23				7.35	June 26, first cutting.	After No. 22.
24	1926	} Feldt (7).....	Germany.....	16.75	July 8, second cutting	After No. 23.
25				19.45	July 7, second cutting	After Nos. 21 and 24.
26				7.93	Sept. 20, second cutting.	After Nos. 22 and 25.
27				10.64	Sept. 20, third cutting	
28				10.24	Sept. 20, third cutting	
29	1928	Ermert (5).....	do.....	10.48	Probably in bloom..	

Soon after this Arendt and Knop collected plants from 80 to 90 inches high, that were past bloom, separated these into two parts, leaves and culms with panicles, and determined the protein in each.

They found 16.50 per cent in the leaves and only 4.35 per cent in the culms with panicles (1, p. 50), but they do not report the proportion of each. If it be assumed that the leaves formed 50 per cent, a value suggested by the data in Table 9, the moisture-free hay would have contained 10.42 per cent protein.

Nearly 20 years later, in 1876, Storer in Massachusetts collected two samples of wild grass (Nos. 5 and 6 in Table 1) shortly before the heads appeared, with the individual plants varying from 24 to 54 inches in height, and found a slightly higher protein content than any previously reported (22, p. 132). The few later analyses in this country, all made 13 to 18 years after 1876, were of wild plants or of plants grown from the seed of these. The experiment stations of Colorado (4, p. 88), Iowa (20, p. 457), South Dakota (19, p. 54), Utah (6, p. 255), and Vermont (12, p. 86) each report one analysis. The percentage of protein found varied from 7.78 to 16.88 per cent (Nos. 7, 8, 9, 11, and 13 in Table 1).

Stebler (21, p. 87) of Switzerland found 6.9 per cent in the hay.

In the report on an investigation of the forage plants of Finland, in 1908, Rindell included analyses of seven samples of *Phalaris arundinacea* (Nos. 14 to 20 in Table 1). From the analysis of two samples from the same meadow, Nos. 18 and 19, he concluded that if the grass had been cut somewhat before bloom it would have been richer in protein. His analyses gave the highest protein values reported from Europe previous to the recent work of Feldt, but these were exceeded by those already reported from Vermont, 15.09 per cent, and from Iowa, 16.88 per cent.

During the following 20 years the grass appears to have received little attention, but in February, 1926, Feldt, of Königsberg, speaking before the Zentral Moor Kommission of Germany, pointed out that new determinations of its protein content should be made and suggested that through breeding and mowing several times in a season the grass could be made a more valuable forage plant than it is at present (7, p. 137). At a meeting of the same organization in December of that year he reported the analysis of eight samples, Nos. 21 to 28 in Table 1 (8, p. 98). The grass was grown on a loamy sand, rich in organic matter, and had been fertilized on April 15, 1926, with 200 kilograms per hectare of Leunasalpeter (equivalent to 290 pounds per acre of sodium nitrate). Three plots or tracts were mowed on different dates: No. 1 on May 19, July 8, and September 20; No. 2 on June 9, July 7, and September 20; and No. 3 on June 26 and September 20, there being no third growth to mow.

The protein content of the first cutting decreased as the age of the grass increased, namely, from 22 per cent on May 19 to 7.35 per cent on June 26. The second cutting, when made at the end of 28 days, showed 19.45 per cent; when made at the end of 50 days, 16.75 per cent; but when delayed for 98 days after the latest first cutting, it fell to 7.93 per cent. The two samples from the third growth, cut 75 days after the second, contained only 10 per cent protein. It should be stated that while a nitrogen fertilizer had been applied to all the plots in the spring there had been no later application. The samples analyzed were not of wild plants, nor from the commercial seed of Germany, but from a leafy strain, Massenauslese, developed by the Mooramt, and Feldt warns against assuming that the above protein

percentages from this strain, rich in leaves and poor in culms, would be found to apply to the wild strains of *Phalaris arundinacea*, which are almost always rich in culms. He expressed the opinion that most of the reed canary grass seed now on the market should not be sown on meadows from which a protein-rich hay is desired. Weber, in his monograph of which the preface is dated July, 1927, states that he had hunted in vain for analyses made of the hay harvested at the proper time, the one coming nearest being that by Rindell (17) mentioned above (No. 18 in Table 1). Evidently he was not acquainted with the paper by Feldt (8) which had been presented before the Zentral Moor Kommission six months before but was not published until much later.

Ermert (5, p. 175), of Königsberg, in a study of the forage value of hays in 1928, reported the protein in the hay from seven grasses all cut at the same stage of maturity, but what that was he does not state. The reed canary grass contained 10.48 per cent protein in the dry matter and the timothy 9.82 per cent.

EXPERIMENTAL DATA

SOURCE OF SEED

The samples of grass analyzed in this study were from plants grown from seed secured from three different sources. The main supply was of the Randowbruch strain of *Phalaris arundinacea*, purchased in the spring of 1927 from the meadow and pasture section of the Verein zur Förderung der Moorkultur im Deutschen Reiche, and referred to below as the German seed. About the same time, through the kindness of A. Kirssanoff, of Leningrad, small quantities of seed were secured from four Russian agricultural experiment stations, namely, those at Marussino, Moscow, Detskoe Selo, and Beresotichosk, the largest amount from the first. Lastly, there was seed from the Lake Madison district, 60 miles southwest of St. Paul, Minn., where a considerable acreage of the grass has been slowly developed from seed purchased by a local farmer from a Wisconsin seed company 30 years ago.

In presenting the results the data are grouped according to the source of the seed, although it was no purpose of the study to attempt the selection of a superior strain. The samples analyzed consisted of the entire aerial portion of the plants, cut off about 3 inches above the surface of the ground, taken to the laboratory, dried at about 50° C., finely ground, and finally dried at 100° C. before analysis. The percentages of crude protein reported in the tables are on the moisture-free basis and not on the hay, which is ordinarily assumed to carry 15 per cent moisture.

GRASS FROM MINNESOTA SEED

On September 27, 1926, a sample of the second growth was collected near Lake Madison from a meadow in which the first growth had been cut early in July, just after the seed had ripened. This aftermath was about 27 inches in height and contained 14.5 per cent protein. None of the plants in this, or any of the second and third cutting samples reported in this paper, showed any sign of forming heads.

AT COON CREEK EXPERIMENTAL FIELDS

Some of the seed that had been gathered from the above field and a small sod were taken to the Coon Creek peat experimental fields, 20 miles northwest of St. Paul, where on October 6, 1926, the sod, divided into four parts, was set out in rows. On the same day part of the seed was planted in five 5-foot rows 36 inches apart, the rest being sown a year later. Plants appeared early in the spring of 1927, and during the summer made a vigorous growth. On two of the rows they were harvested twice that season. On the first date, August 3, they were about 27 inches high, had not yet formed heads, and contained 17.31 per cent protein, while the second growth, cut on November 4, was about 12 inches high and contained 13.94 per cent protein. The plants in the three remaining rows were cut only once, on November 4, by which time they had reached a height of about 33 inches, and put forth a few heads, and carried 11.06 per cent protein. (Table 2.)

TABLE 2.—*Protein content of reed canary grass from Minnesota seed, grown in rows 36 inches apart on Coon Creek peat experimental fields, 1927-28*

Reference No.	Date of sampling	Protein content on a dry basis	Cutting	Average height of plants	Stage of maturity	Remarks
	1927	<i>Per cent</i>		<i>Inches</i>		
2	Aug. 3	17.31	First....	27	No sign of heads.....	Seed sown in October, 1926.
3	Nov. 4	13.94	Second....	12do.....	Aftermath of No. 2.
4do.....	11.06	First....	33	A few heads formed....	Sown at same time as No. 2.
	1928					
5	June 7	15.88do.....	38	A third of heads out....	From same rows as No. 4, mowed only once in 1927; seed sown in October, 1926.
6	July 23	21.94	Second....	18	No sign of heads.....	
7	June 14	15.75	First....	45	Most of heads out....	
8	Aug. 17	14.25	Second....	25	No sign of heads.....	
9	June 27	10.69	First....	54	All in head.....	From same rows as Nos. 2 and 3, mowed twice in 1927; seed sown in October, 1926.
10	Aug. 17	13.00	Second....	25	No sign of heads.....	
11	June 7	13.81	First....	-----	No heads out.....	
12	July 23	20.81	Second....	15	No sign of heads.....	
13	June 14	14.82	First....	36	A few heads out.....	Seed sown in October, 1927.
14	June 27	10.56do.....	51	Beginning to bloom....	
15	Aug. 17	14.06	Second....	16	No sign of heads.....	
17	July 21	20.00	First....	23	No sign of heads, south plot.	
18	Aug. 17	15.66	Second....	29do.....	From sods transplanted in October, 1926; first growth, 50 inches high, cut July 6 after seed had ripened.
19	July 21	19.92	First....	23	No sign of heads, north plot.	
20	Aug. 17	21.19	Second....	27do.....	
21do.....	17.19do.....	23	No sign of heads.....	

In 1928 those of the rows from seed sown in October, 1926, that had been mowed only once in 1927, were divided into three lots and the first cutting made on June 7, 14, and 27, respectively. On the first date about 30 per cent of the heads had appeared, on the second about 60, while on the last the grass was fully in head and beginning to bloom. The first sample contained 15.88 per cent protein, the second practically the same—15.75 per cent—but the third only 10.69. (Table 2.)

The rows from seed sown at the same time as the preceding, but mowed twice in 1927, were similarly divided into three lots and harvested on the same three successive dates. The grass from the

first contained 2 per cent and that from the second 1 per cent less protein than the corresponding samples from the rows cut once in 1927, but on the latest date there was practically no difference.

The second cutting from the parts first mowed on June 7 was made at the end of 46 days, and contained 21.94 and 20.81 per cent protein, while that from the other lots, all sampled on August 17, contained only from 13.00 to 14.25 per cent.

On October 19, 1927, the remainder of the seed secured from the Lake Madison district in 1926 was sown at Coon Creek in rows 3 feet apart on two small plots adjacent to the first. No plants appeared above the surface until the following spring. On July 21, when the plants were about 23 inches high, but showed no sign of heading, both plots were mowed. The percentages of protein in the two samples were 20.00 and 19.92. A second cutting was made on August 17, by which time the aftermath was taller than the plants had been when mowed four weeks before. The protein content on one plot was 15.66 and on the other 21.19 per cent, with an average of 18.42. The difference of 5.5 per cent between the two, which had received the same treatment, would suggest that there was a more liberal supply of available nitrogen in the soil of one of the plots.

The transplants were not sampled in 1927; in 1928 the first growth was allowed to ripen seed and the straw was cut on July 6, but no analysis of it was made. The aftermath, cut on August 17, was 23 inches high and contained 17.19 per cent protein. (No. 21 in Table 2.)

On the whole, the protein content of the 20 samples of grass reported in Table 2 was high, averaging 15.88 per cent for all, with a maximum of 21.94 and a minimum of 10.56. The average for all samples of the first cutting was 14.98 and of the second, 16.89 per cent. No nitrogen fertilizer had at any time been applied, but the soil is naturally so rich in available nitrogen that cultivated and grain crops on it respond to nitrogen fertilizers only when the soil is cold, as early in the spring. Furthermore, the plants had the advantage of being in short rows 3 feet apart and surrounded by bare fallowed soil. The spaces between the rows also had been kept free from all vegetation except the underground stems of the reed canary grass. The general effect of these conditions was to assure the grass an unusually liberal supply of nitrogen.

IN THE LAKE MADISON DISTRICT

As previously mentioned, there is a considerable acreage of reed canary grass meadow on farms in the vicinity of Lake Madison, Minn. It is also found growing along the public roads, being especially abundant where roads cross tracts of peat or muck. The meadows, on all of which it had been sown broadcast, in most cases from three to six years before, have a peat or muck soil, well provided with lime and with a supply of available nitrogen at least as abundant as that of the peat at Coon Creek. In the season of 1928, 26 samples of first, second, and third growth from this district were analyzed. (Table 3.) The first 10 samples were collected on June 17, when in most places the grass was more or less headed out but not yet in bloom; the next 7 on June 29, when nearly all the meadows were in bloom; the remaining 9 represent the second or third cutting.

TABLE 3.—*Protein content of reed canary grass grown in the Lake Madison district in 1928*

[The meadows had been seeded three years or more]

Ref- erence No.	Date of sam- pling	Protein content on a dry basis	Cutting	Average height of plants	Stage of maturity	Remarks
		<i>Per cent</i>		<i>Inches</i>		
22	June 17	13.19	First.....	19	A few heads out.....	Farm A, field 1, very thick stand.
23	do	10.69	do	24	do	Do.
24	do	14.22	do	36	All in head.....	Farm A, field 1, adjacent to corn.
25	do	14.75	do	29	A few heads out.....	Farm A, field 2, younger meadow.
26	do	12.06	do	28	All in head.....	On highway beside farm A.
27	do	12.00	do	38	do	Farm B, meadow.
28	do	11.75	do	36	do	Farm C, meadow.
29	do	13.00	do	24	do	Isolated clump on roadside.
30	do	15.25	do	34	do	Do.
31	do	10.00	do	48	do	In roadside ditch.
32	June 29	8.56	do	46	In bloom.....	Farm D, meadow.
33	do	9.13	do	46	do	Farm E, meadow.
34	do	11.88	do	48	do	Farm B, meadow.
35	do	14.50	do	48	do	Farm F, meadow.
36	do	8.00	do	48	do	Farm F, another meadow.
37	do	10.31	do	48	do	Farm G, meadow.
38	do	15.75	do	30	Partly in head.....	Farm H, meadow.
39	July 25	19.75	Second..	13	No sign of heads.....	Farm B, unfertilized.
40	do	19.63	do	13	do	Farm B, F supplied on June 29.
41	do	18.56	do	13	do	Farm B, P+K supplied on June 29.
42	do	18.44	do	13	do	Farm B, N+P+K supplied on June 29.
43	Aug. 20	13.00	do		do	Farm B, unfertilized.
44	Sept. 25	15.06	do		do	Farm I, first growth, cut for seed.
45	Oct. 1	25.25	Third..	11	do	Farm B, second growth, mowed August 20.
46	do	22.13	do	12	do	From roadside near farm B.
47	(1)	14.56	Second..		do	From farm I.

¹ About October 1.

On the oldest field sampled, a small shallow bog that had been seeded in 1919, samples were taken from three places, the first where the stand was very thick and the growth comparatively short, about 19 inches, with only an occasional head appearing, the second from an equally thick growth with plants about 5 inches taller and with an even smaller number in head. (Fig. 1, A and B.)

The third sample was of plants from the extreme edge of the meadow, forming the border of a field of corn that had been kept free of weeds and so was equivalent to a fallow. These plants were much darker in color, a foot taller than the preceding, and all in head, although not yet in bloom. (Fig. 1, C.) Notwithstanding their being more mature they carried from 1 to 3.5 per cent more protein than the preceding two samples. No. 25 (fig. 1, D) was taken as representative of another and larger field on the same farm, also on peat soil, but seeded five or six years after the first. The plants, about a tenth in head, were taller than those of No. 23 (fig. 1, B), but the protein content was higher than in any of the preceding three; that is, 14.75 per cent. A few hundred yards from the first meadow, on mineral soil forming the shoulder of a highway, an isolated clump of the grass was found with bare soil extending from 3 to 5 feet on all sides. On this (fig. 1, E) the heads were fully out but not in bloom, and the protein content was 12.06 per cent.

Nos. 27 and 28, with the plants fully in head, were taken from meadows on two farms a few miles distant. They had a protein content of 12 and 11.7 per cent, similar to that of plants in the thick interior of the first meadow. On a peaty roadside near the last field there were two isolated clumps of the grass, both fully in head and very different in appearance, although only 5 feet apart. One contained 13 per cent and the other 15.25 per cent of protein. The tallest plants seen in the district were found growing near those on peat soil in a roadside ditch. They were 4 feet high, fully headed out, but not

in bloom, and contained only 10 per cent of protein (No. 31 in Table 3).

The samples collected on June 29 were from seven farms, each sample taken as representative of the crop on the whole meadow. On part of the fields haying was in progress at the time, and on all except the last, No. 38, the plants were in full bloom. The protein content ranged from 8 to 15.75 per cent. No. 38 had a protein content of 15.75 per cent, but in this the plants were only partly in head, the field probably having been pastured early in the season.

On June 29 various fertilizers were added to plots laid out on a meadow from which the hay had just been removed and from which a sample (No. 34) was taken. These plots were sampled on July 25, when the second growth was 25 inches high. The protein content varied from 18.44 to 19.63 per cent.



FIGURE 1.—Samples of first-growth reed canary grass collected on June 17 from a farm in the Lake Madison district, Minn. A (No. 22). From thick stand in interior of an old meadow; average height, 19 inches; protein content, 13.19 per cent. B (No. 23). From thick stand in interior of an old meadow; average height, 24 inches; protein content, 10.69 per cent. C (No. 24). From edge of same meadow bordering on clean, cultivated land; average height, 36 inches; protein content, 14.52 per cent. D (No. 25). From a younger meadow; average height, 29 inches; protein content, 14.75 per cent. E (No. 26). From shoulder of the adjacent highway; average height, 28 inches; protein content, 12.06 per cent. Numbers correspond with those in Table 3

A sample of the third growth on this field, taken on October 1, when the grass was about 12 inches high, contained 25.25 per cent protein, while a sample of about the same height taken from a near-by roadside contained 22.13 per cent (Nos. 45 and 46).

The average protein content of the 10 samples taken on June 17, while the grass was only partly in head, was 12.69 per cent, that of the 7 taken 12 days later, when the grass was in bloom, was 11.16 per cent, while the 9 samples of aftermath averaged 18.49 per cent.

This would give an average of 14.29 per cent for all 26 samples from the Lake Madison district. The average protein content of the crop for the entire season would be much below 14 per cent because of the comparatively small proportion that the aftermath formed.

GRASS FROM RUSSIAN SEED

The four lots of Russian seed mentioned above were planted at Coon Creek on June 15, 1927, in rows 36 inches apart. On November 4 the Marussino grass was sampled and found to contain 12.75 per cent protein. In 1928 a part of the rows from this source and all of those from the other Russian sources were allowed to ripen seed, not being mowed until July 6. The plants from all four lots appeared very much alike. The protein content of the first growth, freed of ripe seed before analysis, was low, ranging only from 6.94 to 8.31 per cent. (Table 4.) The aftermath, sampled on August 17, contained from 14.25 to 18.63 per cent. The relative richness in protein of the second cutting bore no distinct relation to that of the first. Thus the Beresotochsk ranked highest in the first cutting and lowest in the second, whereas the Marussino, which was next to the lowest in the first cutting, was highest in the second.

TABLE 4.—*Protein content of reed canary grass from Russian seed, sown in rows 36 inches apart, on Coon Creek peat experimental fields on June 15, 1927*

Reference No.	Date of sampling	Protein content on a dry basis	Cutting	Average height of plant	Stage of maturity	Source of seed
		<i>Per cent</i>		<i>Inches</i>		
48	1927 Nov. 4	12.75	First		No sign of heads	
49	1928 July 6	7.19	do	58	Ripe straw, without seed.	Marussino Experiment Station, Tambov Government.
50	Aug. 17	18.63	Second	22	No sign of heads	Do.
51	July 6	8.31	First	58	Ripe straw	Beresotochsk Experiment Station, Poltava Government.
52	Aug. 17	14.25	Second	20	No sign of heads	Do.
53	July 6	6.94	First	58	Ripe straw	Plant breeding station, Detskoe Selo, Leningrad Government.
54	Aug. 17	15.88	Second	16	No sign of heads	Do.
55	July 6	7.44	First	58	Ripe straw	State institute of meadow management, Moscow Government.
56	Aug. 17	17.44	Second	20	No sign of heads	Do.

Three rows of Marussino plants were mowed on June 18, no sample being saved for analysis, and divided into 11 parts for second and third cuttings. Beginning July 13, these were sampled and mowed the second time at 3-day or 4-day intervals, the latest mowing being on August 24. The third cutting on each was made from 49 to 53 days after the second, the earliest being on September 4 and the latest on October 9. Accordingly, samples of the aftermath were collected about twice a week during the last three months of the growing season. (Table 5.) The protein content of the 11 samples of second growth of Marussino averaged 18.86 per cent and ranged from 16.31 to 22.56 per cent without any distinct dependence upon the time of the cutting, the minimum being found at the end of 46 days and the maximum 10 days later. The growth of the plants was not vigorous. At the end of 26 days they were about 16 inches high,

and six weeks later they were only 6 inches taller. With the third growth the average protein content, 15.56 per cent, was about 3 per cent lower than with the second and the variations were narrower.

TABLE 5.—*Protein content of reed canary grass collected in 1928 from Marussino and Randowbruch seed, sown in rows 36 inches apart, on Coon Creek peat experimental fields on June 15, 1927*

[All of Nos. 71 to 114 from rows mowed first time on June 18]

Reference No. of—		Date of sampling	Protein in hay from—		Cutting	Average height of plants from—		Stage of maturity	Growth period
Russian seed	German seed		Russian seed	German seed		Russian seed	German seed		
			Per cent	Per cent		Inches	Inches		Days
57	58	May 28	17.75	19.31	First			No sign of heads	128
59	60	June 4	13.12	15.40	do			do	35
61	62	June 7	12.35	12.91	do	36	29	No heads out	38
63	64	June 14	11.46	10.38	do	46	34	Heads just breaking through	45
65	66	June 21	8.30	10.47	do	53	45	All in head	52
67	68	June 28	7.80	9.71	do	58	54	In full bloom	59
69	70	July 6	6.57	7.47	do	58	58	Seed ripened and fallen	66
71	72	July 13	21.13	18.23	Second	16	12	No sign of heads	25
73	74	Sept. 4	15.43	15.06	Third	14	15	do	52
75	76	July 17	19.56	17.88	Second			do	29
77	78	Sept. 7	15.69	16.06	Third	13	11	do	51
79	80	July 20	17.25	16.50	Second			do	32
81	82	Sept. 11	15.98	16.31	Third	12	13	do	53
83	84	July 24	16.31	15.87	Second			do	36
85	86	Sept. 14	14.69	14.38	Third	12	13	do	52
87	88	July 27	17.25	14.19	Second			do	39
89	90	Sept. 18	15.31	14.50	Third	10	10	do	53
91	92	Aug. 3	22.56	14.50	Second			do	46
93	94	Sept. 21	16.44	16.19	Third		12	do	49
95	96	Aug. 7	18.56	13.06	Second		10	do	50
97	98	Sept. 25	14.88	16.75	Third		12	do	49
99	100	Aug. 10	20.81	14.31	Second		12	do	53
101	102	Sept. 28	15.06	16.88	Third			do	49
103	104	Aug. 14	18.88	13.69	Second			do	57
105	106	Oct. 2	14.38	15.94	Third			do	49
107	108	Aug. 17	13.63	12.81	Second	22	17	do	60
109	110	Oct. 5	14.69	16.56	Third			do	49
111	112	Aug. 24	17.19	12.08	Second	22	19	do	67
113	114	Oct. 9	18.62	17.94	Third			do	46
All second cuttings:									
Average			18.86	14.83		20	14	do	
Maximum			22.56	18.23		22	19	do	
Minimum			16.31	12.08		16	10	do	
All third cuttings:									
Average			15.56	16.05		12	12	do	
Maximum			18.62	17.94		14	15	do	
Minimum			14.38	14.38		10	10	do	

¹ In computing the period up to the first cutting it has been assumed that growth began on May 1.

TABLE 6.—*Yield, protein content, and average height of reed canary grass from Marussino seed, sown June 15, 1927, in rows 36 inches apart, at Coon Creek, and mowed in 1928*

Reference No.	Date of mowing	Stage of maturity	Average height of plants	Protein content on a dry basis	Yield per acre	Length of row harvested	Remarks
			Inches	Per cent	Tons	Feet	
61	June 7	No heads out	36	12.35	2.7	9	First growth
63	June 14	Heads just appearing	46	11.46	2.9	9	Do.
65	June 21	All in head	53	8.30	2.9	9	Do.
67	June 28	In full bloom	58	7.80	2.9	9	Do.
69	July 6	Seed ripened and fallen	58	6.57	3.6	9	At end of row adjacent to fallow.
107	Aug. 17	No sign of heads	22	18.63	1.5	45	Aftermath of hay mowed June 18.

The few data on yields secured serve only to indicate what may be regarded as the maximum yields obtainable under the circumstances. (Table 6.) Only in the case of the Marussino plants was a weighing made of samples from a row in which there was a thick stand of

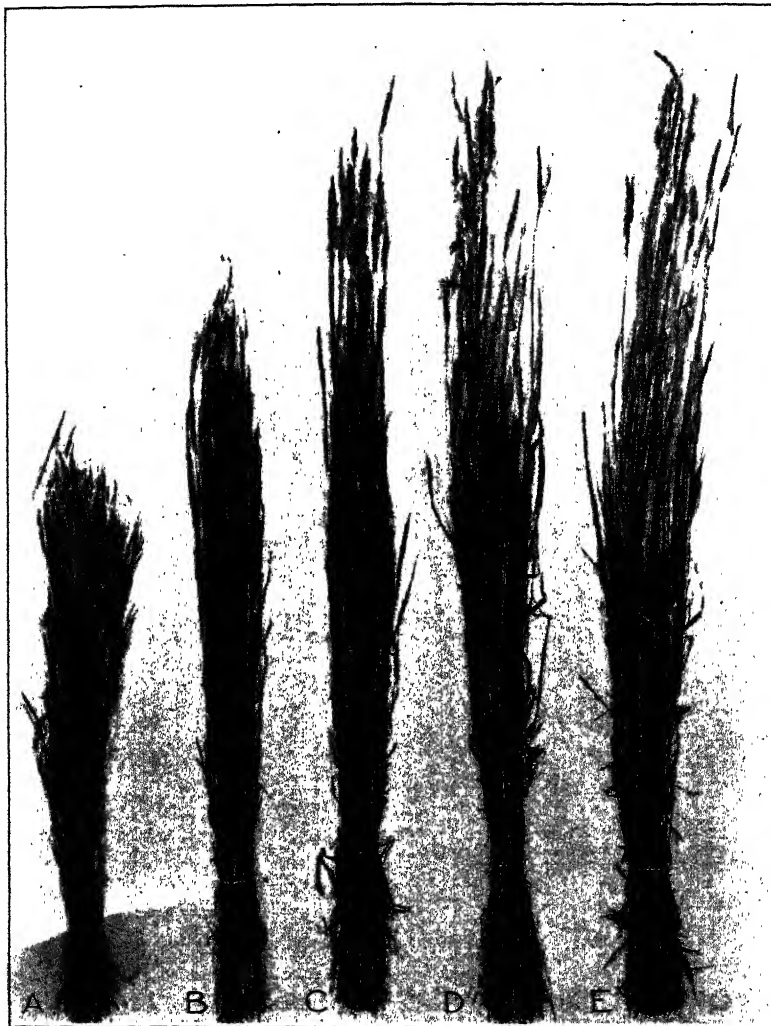


FIGURE 2.—Samples of first growth of reed canary grass sown at Coon Creek, in June, 1927, and cut on different dates in 1928. A (No. 61), Average height, 36 inches; protein content, 12.35 per cent. B (No. 63), Average height, 46 inches; protein content, 11.46 per cent. C (No. 65), Average height, 53 inches; protein content, 8.3 per cent. D (No. 67), Average height, 58 inches; protein content, 7.8 per cent. E (No. 69), Average height, 58 inches; protein content, 6.57 per cent. Numbers correspond with those in Table 6

plants, and on both sides of which there were rows which also had a thick stand. In the first five samplings of the first growth the grass from a 9-foot section of the row was weighed, but the last section, cut on July 5, being at the end of the row next to a fallow pathway,

had a larger area to draw upon for moisture and nutrients. The yield during the 3-week period, June 7 to 28, remained practically stationary. On a 45-foot row, in an equally satisfactory position for data on yields, and from which the first growth had been removed without weighing on June 18, the second cutting, on August 17, gave a yield of 1.5 tons per acre. If it be assumed that the yield of the first cutting on this was the same as that on the 9 feet of row cut on June 14, the total yield of hay from the two cuttings would amount to 4.4 tons per acre. In the computation of the yields, the rows being 3 feet apart, it has been assumed that the plants in the 9 feet of row occupied 27 square feet. The general appearance of the plants tied in bunches after sampling may be seen from Figures 2 and 3. The same scale is used in both illustrations.

GRASS FROM GERMAN SEED

At Coon Creek the first seeding was made on June 15, 1927, in rows 36 inches apart. Two days later a near-by plot was seeded broadcast

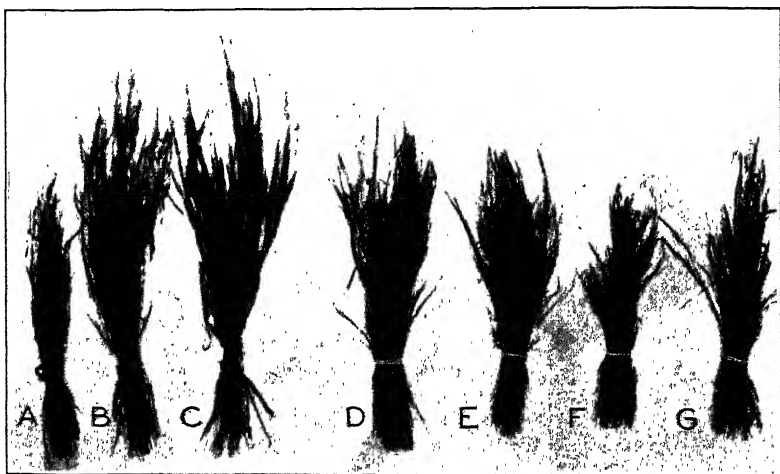


FIGURE 3.—Samples of aftermath of reed canary grass sown at Coon Creek, in June, 1927, and cut at different dates in 1928. A (No. 72), second cutting on July 13 after a growth period of 25 days; average height, 12 inches; protein content, 18.23 per cent. B (No. 108), second cutting on Aug. 17 after a growth period of 60 days; average height, 17 inches; protein content, 12.81 per cent. C (No. 112), second cutting on Aug. 24 after a growth period of 67 days; average height, 19 inches; protein content, 12.08 per cent. D (No. 73), third cutting on Sept. 4 after a growth period of 52 days; average height, 14 inches; protein content, 15.43 per cent. E (No. 81), third cutting on Sept. 11 after a growth period of 53 days; average height, 12 inches; protein content, 15.98 per cent. F (No. 89), third cutting on Sept. 18 after a growth period of 53 days; average height, 10 inches; protein content, 15.31 per cent. G (No. 98), third cutting on Sept. 25 after a growth period of 49 days; average height, 12 inches; protein content, 16.75 per cent.

at the rate of 20 pounds per acre, as advised by Piper (15, p. 231). More than 90 per cent of the seed germinated and a very thick stand resulted. On the Page low-lime peat experimental field, 70 miles north of St. Paul, the grass was seeded broadcast on July 9, at the same rate, and an equally thick stand was obtained. About a year later, June 29, 1928, a second seeding was made on the same field but in rows 16 inches apart.

On November 4, 1927, a sample of the grass from the rows seeded on June 15 was taken and found to contain 15.25 per cent protein (No. 115). In 1928 samplings were made on the same 29 dates as the

Russian plants. (Table 5.) Up to the time of full bloom the German plants were shorter and richer in protein than the Russian. With the second cutting there was a practically steady decline from 18.23 per cent at the end of 25 days to 12.08 at the end of 67 days, although with the Russian no similar regular decline was found. The third cutting showed a higher average, 16.05 per cent, than the second and a higher minimum, 14.38, but a similar maximum 17.94. The average for the third cutting was 0.5 per cent higher than for the Russian, whereas that for the second cutting had been 4.1 per cent lower.

All the plots at both Coon Creek and Page had been fertilized liberally in 1927, and again in 1928, with 45 per cent superphosphate and 50 per cent potassium chloride. On parts of the broadcast plots at both places ammonium sulphate was applied in 1928, at Page on May 15 at the rate of 200 pounds per acre, and at Coon Creek on May 29 at the rate of 100 pounds per acre. On no part of these broadcast plots, except the borders, next to the clean cultivated roadways, did the grass make a satisfactory growth. At both places the portion given nitrogen fertilizer was taller and had a much darker color. Samples were taken from the interior of the plots at intervals in 1928, beginning at Page on June 1 and at Coon Creek on July 17 and continuing at both places up to the time of mowing the entire plots—July 21 at the former and August 7 at the latter. At neither place after the first mowing was there any appreciable growth, except along the bare roadway at Page, or any distinct effect of the ammonium sulphate application.

TABLE 7.—*Protein content, yield, and average height of reed canary grass in 1928 on plots sown broadcast with German seed in 1927.*

[Part of each plot was given an application of ammonium sulphate in 1928; at Page, 200 pounds per acre on May 15 and at Coon Creek, 100 pounds per acre on May 29]

AT PAGE

Reference No. of samples from plots—		Date of sampling	Protein content on a dry basis		Yield of hay per acre		Average height of plants	
Without N fertilizer	With N fertilizer		Without N fertilizer	With N fertilizer	Without N fertilizer	With N fertilizer	Without N fertilizer	With N fertilizer
			Per cent	Per cent	Tons	Tons	Inches	Inches
116	117	June 1	10.81	15.13	0.5	0.7	(*)	(*)
118	119	June 8	10.69	13.13	.5	.7	(*)	(*)
120	121	June 19	10.84	11.25	-----	.7	11	15
122	123	June 23	10.00	14.44	.6	.8	12	19
124	125	June 30	10.69	12.56	.6	1.2	12	19
126	127	July 7	10.88	10.63	-----	-----	14	24
128	129	July 15	9.81	9.56	.4	1.1	15	25
130	131	July 21	9.81	9.75	.4	1.5	16	25

AT COON CREEK

132	133	July 17	8.75	9.38	-----	0.9	(*)	(*)
134	135	July 20	7.94	9.56	0.8	.9	(*)	(*)
136	137	Aug. 3	8.25	7.75	.9	1.2	(*)	(*)
138	139	Aug. 7	7.63	7.63	.9	1.5	18	18

* No record of height.

At Coon Creek, both with and without nitrogen fertilizer, the yield and the protein content of the hay were low (Table 7), although the nitrogen application increased the former and, in the case of the earlier samplings, somewhat raised the latter. On the portion of the plot at Page given no nitrogen fertilizer the plants were short, the yield was light, and the protein content was not high at any time during the season. On the part given ammonium sulphate the growth was much taller and the yield several times as heavy. The protein content, which was 50 per cent the higher on the nitrogen-treated plot on June 1, was practically the same on both during the last three weeks.

The irregularity of the yields from the small plots at Page on successive dates, from June 8 to July 15, may be attributed to the small area, 1 square yard, harvested on each date except the last.

The scattered plants adjacent to the roadways at the ends of the plots at Page made a fairly vigorous growth after the mowing of July 21. Sufficient of these for analysis were collected at weekly intervals, beginning August 18. The protein content was found to decline gradually from 21.5 per cent on August 18 to 10.5 per cent on October 6. (Table 8.)

TABLE 8.—*Protein content and average height of reed canary grass at Page cut during latter part of the season of 1928*

[The crop from the 1927 seeding consisted of the second growth * of scattered plants from the edge of a plot sown on July 9, 1927, and given no nitrogen fertilizer; that of the 1928 seeding was from rows 16 inches apart sown on June 29, and given 200 pounds per acre of ammonium sulphate on August 17]

Reference No.		Date of sampling	Protein content on a dry basis of grass from—		Average height of plants from—	
1927 seeding	1928 seeding		1927 seeding	1928 seeding	1927 seeding	1928 seeding
			<i>Per cent</i>	<i>Per cent</i>	<i>Inches</i>	<i>Inches</i>
141	149	Aug. 18.....	21.50	23.31	14	14
142	150	Aug. 25.....	19.75	20.83	15	16
143	151	Sept. 1.....	17.25	18.81	18	19
144	152	Sept. 8.....	13.37	15.00	17	19
145	153	Sept. 15.....	12.69	14.31	17	21
146	154	Sept. 22.....	12.56	11.81	15	20
147	155	Sept. 29.....	12.19	12.44	-----	23
148	156	Oct. 6.....	10.50	10.38	17	24

* The first growth of these scattered plants was mowed July 21, when they were all in head and about 28 inches tall. The protein content was 10.62 per cent. (Reference No. 140.)

At Page, on June 29, 1928, Randowbruch seed was sowed in rows 16 inches apart, and seven weeks later, August 17, ammonium sulphate was applied at the rate of 200 pounds per acre. The grass, which made a very vigorous growth, was sampled at weekly intervals, beginning on August 18. It increased in height from 14 inches at the first sampling to 24 at the last, while the protein content fell from 23.31 to 10.38 per cent. (Table 8.) The yield per acre of hay, determined on October 6, amounted to 2.1 tons.

The protein content of the second growth of the scattered old plants with much fallow soil to draw upon was very similar to that of the first growth of the young plants that had been given ammonium sulphate, in both declining from over 21 per cent on August 18 to less than 11 per cent by October 6. (Table 8.)

DISTRIBUTION OF PROTEIN IN AERIAL PORTIONS OF PHALARIS ARUNDINACEA

Hosford was the first to report the protein content of leaves of *Phalaris arundinacea* separated from the rest of the plant. In his analyses he found 7.06 per cent in the leaves, 2.81 per cent in the joints, and only 0.5 per cent in the stalks without joints or leaves (9, p. 83). Arendt and Knop, using plants past bloom, found 16.5 per cent protein in the leaves and 4.35 per cent in the culms, including the panicles (1, p. 50). Feldt, referring to this paper, points out that the larger the proportion of culms in a strain of the grass the less will be its value, and that this indicates both the best time for mowing and in what direction breeding should be conducted.

Several months after the analyses reported in Tables 2 to 8 had been completed it was decided to devote some attention to the protein content of the leaves. No unground portion remained of part of the samples, but in the case of many duplicates of the original had been saved for reference as to height and stage of development. Part of these were found to have been injured to such an extent that they were unsatisfactory for analysis, but over 40 samples have been separated into either two or three parts, culms, leaves, and also panicles, wherever the last formed as much as 2 per cent of the dry samples. Where the panicle formed less it has been included with the leaves. In the case of each separate part a determination was made of the proportion which it formed of the total dry matter as well as of the protein content. (Table 9.)

With a smaller number the leaves were carefully divided into blade and sheath, and similar determinations were made. (Table 10.) For some samples data are reported in both tables. The protein content of the entire grass (Table 9) or of the entire leaf (Table 10) has been computed from the data on the different parts and is reported in the second column. Where a well-preserved specimen of the original sample was available for the separation the computed protein content has been substituted in the earlier tables for the similar, but not identical, value that had earlier been obtained by using the entire aerial portion of the plants. In such cases the same reference numbers are used. Thus, Nos. 61 to 70 refer to the same samples of grass in Table 9 that they do in Table 5. Where a sample used for a separation had suffered loss by shattering, as in the case of No. 31, or was not a part of the original samples analyzed but was simply taken from the same plot on the same day, as with No. 69-A, the letter A has been added to the original number.

TABLE 9.—*Distribution of protein and of dry matter in the aerial portion of Phalaris arundinacea grown in the crop season of 1928*
AT COON CREEK, FROM MARUSSINO SEED

Reference No.	Protein content of grass	Percentage protein content of—			Percentage of dry matter in—			Ratio: N in culms N in leaves	Ratio: Weight of leaves Weight of culms	Date of sampling	Cutting	Average height of plants (inches)
		Culms	Leaves	Panicles	Culms	Leaves	Panicles					
61	Per cent	7.04	16.88	—	46	54	0	2.4	1.2	June 7	First	36
62	12.35	6.92	13.82	18.60	40	41	9	2.2	.8	June 14	do	46
63	10.38	5.73	13.62	21.64	35	36	9	1.7	.7	June 21	do	53
64	7.80	4.55	11.60	18.52	37	33	8	2.6	.5	June 28	do	58
65	6.57	4.45	8.51	16.08	37	33	8	1.6	.5	July 7	do	58
66	6.68	2.85	10.41	(¹)	55	43	0	4.0	.8	July 17	do	58
68A	15.20	3.20	10.41	—	26	74	0	6.1	2.8	Aug. 4	Second	16
54A	15.43	6.15	18.52	—	25	73	0	3.0	3.0	Sept. 4	Third	14
73	15.98	6.84	18.56	—	22	78	0	2.7	3.5	Sept. 11	do	12
81												

AT COON CREEK, FROM RANDOWBRUCH SEED

62	12.01	7.39	14.73	30.52	29	69	2	2.0	2.4	June 7	First	29
64	11.46	5.47	14.12	21.90	37	56	11	2.6	1.5	June 14	do	34
65	10.77	5.02	12.97	17.77	41	48	11	2.3	1.2	June 21	do	34
68	6.71	3.24	14.50	14.50	49	47	4	2.2	1.0	June 28	do	45
70	6.04	3.24	13.72	5.38	49	48	2	3.0	1.0	July 6	do	54
72	18.23	6.64	20.27	—	15	85	0	2.1	1.0	July 13	do	58
108	12.81	3.01	15.56	—	20	80	0	2.1	3.6	Aug. 13	Second	12
112	12.08	3.08	15.64	—	20	80	0	2.2	2.3	Aug. 24	do	17
157	17.60	7.88	19.50	—	17	83	0	2.5	4.9	Sept. 20	Third	19
												11

AT COON CREEK, FROM AMERICAN SEED

13	14.82	8.34	10.22	22.98	45	43	12	2.3	1.0	June 14	First	36
14	10.56	5.20	16.49	14.41	51	41	8	3.2	.8	June 27	do	51
19	19.92	11.92	23.51	—	31	69	0	2.0	2.2	July 21	do	23
18	15.66	6.09	20.81	—	35	65	0	3.4	1.9	Aug. 17	Second	29

AT PAGE, FROM RANDOWBRUCH SEED

	19.09 13.80	6.50 5.62	23.20 15.36	25 16	75 84	0 0	3.6 2.7	3.0 5.2	Sept. 1 Oct. 6	First do	19 21
151A 156A											
IN MADISON LAKE DISTRICT, FROM AMERICAN SEED											
22	13.19	6.02	15.01	26	72	2	2.6	2.8	June 17	First	19
23	10.69	5.20	12.83	28	72	6	2.5	2.6	do	do	24
24	14.22	7.67	17.34	45	44	11	2.5	1.0	do	do	36
25	12.06	5.80	15.07	31	68	1	2.4	2.2	do	do	30
26	12.06	5.80	15.07	32	66	2	2.5	2.1	do	do	28
27A	11.69	6.41	16.85	50	50	(1)	2.6	1.0	do	do	38
28A	13.19	6.01	17.02	49	41	10	2.5	1.8	do	do	36
31A	8.52	4.85	13.04	56	43	(1)	2.7	1.1	do	do	48
34A	11.58	5.90	16.63	47	53	1	2.8	1.1	do	do	48
37A	10.05	6.55	16.28	64	36	(1)	2.5	1.0	do	do	48
38A	10.87	11.92	23.11	20	71	(1)	1.9	2.5	do	do	30
39A	10.86	8.82	22.97	22	78	(1)	2.6	3.6	July 25	Second	13

1 Not included, part having been lost.

TABLE 10.—Distribution of protein and of dry matter in the leaves of *Phalaris arundinacea* grown in the crop season of 1928

Refer- ence No.	Percentage protein content of—			Percentage of dry matter of leaf in—		Ratio: N in blade N in sheath	Ratio: Weight of blade Weight of sheath	Date of sampling	Cutting	Average height of plants (inches)
	Entire leaf	Sheath	Blade	Sheath	Blade					
61A	15.04	7.50	20.07	40	60	2.7	1.5	June 7	First	36
27A	16.85	8.44	22.70	41	59	2.7	1.4	June 17	do	38
37A	10.28	9.12	22.13	45	55	2.4	1.2	June 27	do	48
14A	15.73	9.40	21.29	47	53	2.2	1.1	July 7	do	51
69A	11.36	6.03	15.56	44	56	2.0	1.3	Sept. 1	do	58
151A	23.29	13.19	26.31	23	77	2.0	3.3	Oct. 6	do	19
156A	14.75	8.12	17.87	32	68	2.2	2.1	Aug. 10	do	24
158	17.21	6.90	20.47	24	76	3.0	3.2	Sept. 17	Second	16
54A	19.41	8.51	23.04	25	75	2.7	3.0	Oct. 6	do	18
143A	23.91	12.81	26.87	21	79	2.1	4.0	Sept. 1	do	17
148A	15.36	0.88	17.50	20	80	2.5	4.3	Oct. 6	do	
157	19.64	9.73	21.96	19	81	2.3		Sept. 20	Third	

In all cases the percentage of protein in the leaves was much larger than in the culms, the ratio for all averaging 2.8. The proportion of the dry weight formed by the leaves varied in the first cutting from 31 to 72 per cent and in the second and third cuttings from 65 to 85 per cent. With the first cutting the proportion fell up to the time of bloom, in the case of the Marussino grass declining from 54 per cent on June 7 to 31 per cent three weeks later. (Nos. 61 and 67 in Table 9.) On each of the different first dates of cutting the Randowbruch grass showed a higher proportion of leaves than the Marussino. The panicles were in most cases richer in protein than the leaves and in all cases richer than the culms, but in none of the samples did they constitute as much as 12 per cent of the whole. The decrease in the proportion of leaves in the grass up to the time of bloom that accompanied advancing maturity of the first growth was not paralleled by as large a decrease in their richness in protein, as may be seen from the fourth column of Table 9.

Within the leaf itself there was a marked difference between the blade and the sheath. The blade formed from 53 to 81 per cent of the dry matter of the leaf. In the samples reported in Table 10 the percentage of protein in the blades is from 2 to 2.7 times as high as in the sheaths. Thus, in general, the blade was found at least twice as rich in protein as the sheath and the entire leaf was more than twice as rich as the culm. Accordingly, the higher the proportion of culm and sheath the lower would be the percentage of protein. As the length of the sheath increases along with that of the culm, the percentage of protein in the hay may be increased both by mowing before the culms and sheaths have reached their full development and by the selection and breeding of strains of the grass that are characterized by short internodes.

PROTEIN CONTENT OF PHALARIS ARUNDINACEA COMPARED WITH THAT OF TIMOTHY

Timothy is at present the most important grass for meadows on northern peat soils, being sown either with alsike and medium red clovers or with alsike alone. For two or three years, and sometimes longer, this practice gives a hay rich in clover, but later the clovers disappear, leaving timothy as a pure stand or in mixture with various volunteer grasses. In order to justify its substitution for timothy on land that is sufficiently well drained, reed canary grass must be shown to be capable of furnishing more protein per acre than the average from a timothy-clover mixture for the first four or five years after seeding.

At both Page and Coon Creek some comparisons were made of the protein content of the timothy with that of reed canary grass. At Page, on a plot near that on which reed canary grass had been sown broadcast in the summer of 1927, timothy was sown at the rate of 12 pounds per acre without admixture with any other seed, and a full stand was secured. In 1928 part of this was given 200 pounds per acre of ammonium sulphate at the same time that this fertilizer was applied to part of the plot of reed canary grass. Beginning on June 1, samples for analysis were taken at approximately weekly intervals from the two parts of both plots. (Table 11.)

TABLE 11.—*Comparison of protein content and yield of reed canary grass and timothy grown with and without nitrogen fertilizer on Page peat experimental field in 1928*

[Both grasses sown broadcast in July 1927]

PROTEIN CONTENT OF MOISTURE-FREE GRASS

Date of sampling or mowing	Without N fertilizer		With N fertilizer	
	Reed canary grass	Timothy	Reed canary grass	Timothy
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
June 1.....	10.81	13.75	15.13	15.07
June 8.....	10.69	13.75	13.13	14.56
June 23.....	10.00	13.31	14.44	11.94
June 30.....	10.69	11.82	12.56	9.96
July 7.....	10.88	9.37	10.63	8.75
July 15.....	9.81	10.87	9.56	7.31
July 21.....	9.81	9.00	9.75	6.56
Average.....	10.38	11.67	12.17	10.59

YIELD PER ACRE OF HAY

	<i>Ton</i>	<i>Ton</i>	<i>Tons</i>	<i>Tons</i>
July 21.....	0.4	1.0	1.5	1.5

YIELD PER ACRE OF PROTEIN

	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
July 21.....	66	153	248	170

On the subplots given no nitrogen fertilizer the timothy was the richer in the four samplings in June, but the poorer in two of the three in July. On the plots given ammonium sulphate the timothy was much the poorer in protein in all the samplings except the first and second. The yield of air-dry hay, mowed on July 21, was alike on the nitrogen-treated portions—1.5 tons per acre—but on the portions given no nitrogen fertilizer there was 1 ton of timothy as compared with only 0.4 ton of reed canary grass. By comparing the yields of protein per acre from the four subplots it will be seen that it was highest on the reed canary grass given nitrogen and lowest on the same grass given none.

At Coon Creek similar plots of the two grasses were sown broadcast early in the summer of 1927. (Table 12.) The timothy used for comparison received no nitrogen fertilizer but made the more rapid growth and the first cutting had been removed before sampling of the reed canary grass was begun. The hay from the latter, mowed on August 7, gave a lower yield but had a somewhat higher protein content than the timothy mowed on July 10.

TABLE 12.—*Comparison of protein content and yield of reed canary grass and timothy grown without nitrogen fertilizer on Coon Creek peat experimental field in 1928*

[Both grasses sown broadcast in summer of 1927. Timothy mowed July 10, but reed canary grass not until August 7]

PROTEIN CONTENT OF MOISTURE-FREE GRASS

Date of sampling or mowing	Timothy	Reed canary grass
	<i>Per cent</i>	<i>Per cent</i>
June 13.....	11.52	-----
June 27.....	9.77	-----
June 30.....	8.02	-----
July 10.....	6.21	-----
July 17.....	-----	7.44
July 30.....	-----	6.74
Aug. 3.....	-----	7.01
Aug. 7.....	-----	6.49

YIELD PER ACRE OF HAY

August 7.....	<i>Tons</i> 1.3	<i>Ton</i> 0.9
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YIELD PER ACRE OF PROTEIN

August 7.....	<i>Pounds</i> 144	<i>Pounds</i> 95
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DISCUSSION OF YIELDS OF DRY MATTER AND PROTEIN

The yields of reed canary grass hay obtained from the broadcast seedings at both Coon Creek and Page were very poor. Where no nitrogen fertilizer was applied the yield for the entire season was less than a ton per acre, and only 1.5 tons where this was used. The applications of phosphate and potash had been liberal, the supply of lime was adequate at both places, naturally so at Coon Creek, and as the result of a liberal marling at Page. At Page the temperatures had been favorable and the rainfall unusually plentiful; but at Coon Creek the rainfall was about 20 per cent below the normal. The distance of the water table below the surface at Coon Creek was almost stationary throughout the season, and at Page it did not vary widely until near the close of the season. (Table 13.) At both places a higher average level would have been more favorable for grasses in general and probably especially so for *Phalaris arundinacea*, but the level that prevailed was very favorable for the nitrification of the peat. In general, the conditions at both places were so favorable for grass that the low yield of hay from the broadcast seeding should not be attributed to unusual weather. It was more probably due to an insufficiency of available nitrogen.

TABLE 13.—*Water level, rainfall, and mean temperature at Coon Creek, Page, and in the Lake Madison district during the crop season of 1928*

EXTREMES OF DISTANCE OF WATER TABLE BELOW SURFACE, IN INCHES

Locality, crop, and manner of sowing	May	June	July	August	September
Coon Creek, German grass broadcast.....	-----	25-34	24-37	21-35	26-38
Coon Creek, Russian and German grass, in rows.....	-----	27-42	27-39	36-43	30-41
Coon Creek, American grass in rows.....	-----	43-46	46-53	46-54	45-53
Page, German grass broadcast.....	27-33	23-34	20-29	13-30	10-24
Page, German grass in rows.....	27-30	15-31	10-22	13-22	6-20

PRECIPITATION, IN INCHES

Coon Creek.....	2.2	2.3	3.1	4.8	3.6
Page.....	.8	4.2	6.3	5.5	3.2
Madison Lake ¹	2.0	2.9	2.0	8.8	1.9

MEAN TEMPERATURE, 0° F.

Coon Creek.....	61	63	72	70	59
Page.....	58	60	69	67	55
Madison ¹	62	63	73	70	59

¹ From record at St. Peter, 12 miles from Lake Madison.

The yield of grass sown in rows 3 feet apart was very good. The Marussino strain at Coon Creek, sown at practically the same time as the near-by broadcast plots of German grass and given the same fertilization, produced nearly three times as much hay as the latter. The first cutting on June 18, with a yield of 2.9 tons per acre, may be assumed to have had the same composition as No. 65, sampled three days later (Table 5), and so to have contained 481 pounds of protein; while the second, mowed on August 17, with 18.6 per cent protein (No. 107), gave a yield of 1.5 tons of hay and so of 558 pounds of protein per acre. A yield of 4.4 tons per acre of hay from a meadow is to be considered high and a yield of 1,039 pounds per acre of protein exceptionally high. The German and American grass in rows grew equally well, but data indicating the yields of these on a field scale were not secured.

There is much difference of opinion as to the suitability of peat soils for *Phalaris arundinacea*. Werner in 1889 stated that although it is especially productive on moist soils it does not succeed on peat (25, p. 117), and Strecker as late as 1914 mentions that it does not love peat soils (23, p. 179). Piper's statement that "the grass is said not to thrive in peaty soils" (15, p. 231) may be based on the opinions of these two authors. In 1892 Von Seelhorst recommended it as a valuable grass on very wet peat meadows, especially on those exposed to flooding, but emphasized the fact that in order to secure hay of good quality the grass should be mowed "before blooming at the end of June" and recommended it only for those meadows that are too wet for the commonly used grasses, such as timothy and meadow fescue (18, p. 137). Bersch in 1912 took the same view (3, p. 251).

Heuser, of Danzig, in 1927 states that it is only within the last few years that it has come to be recognized that *Phalaris arundinacea* is one of the most valuable grasses for the peat lands, mentioning that it is especially exacting in its demands for nitrogen (11, p. 139).

Weber considers the character of the soil to be of little importance for the growth of this grass provided the moisture supply and its oxygen content are favorable and there is an abundance of available plant nutrients, either naturally present, supplied in the water, or applied as fertilizers. It grows on sand, loam, marshy clay, peaty soil, high-lime peat (Niedermoor), low-lime peat (Hochmoor) to which lime has been applied, on moderately acid to neutral soils, and on chalk (24, p. 16).

SUMMARY AND CONCLUSIONS

Previous determinations of the crude protein content of *Phalaris arundinacea*, about 30 in number, covering a period of 80 years, and representing 5 European countries and 6 different States in this country, show a great range, with a maximum of 22 per cent and a minimum of 3.44 per cent of the moisture-free grass.

Protein determinations are reported on upward of 150 samples of the grass grown on peat soils in Minneosta, using plants from Russian and German as well as American seed. In the case of more than 30 of these samples the plants were separated into leaf, culm, and panicle, the weight and protein content of each being determined, while with 12 samples the leaves were divided into blade and sheath for analysis.

The highest percentage of protein found in the entire grass, on a moisture-free basis, was 25.2 and the lowest 6.6. It ranged in the culms from 2.8 to 11.9 per cent, in the leaves from 8.5 to 23.5, and in the panicles from 9.4 to 30.5. The maximum found in the culms was higher than the minimum in the leaves, but in the case of every sample there was a much higher percentage in the leaves than in the culms, generally from two to three times as much. The leaves formed from 31 to 72 per cent of the dry matter in the first cutting and from 65 to 85 per cent in the aftermath. With advancing maturity of the first growth up to the time of bloom there was a rapid fall in the proportion of the total dry matter formed by the leaves without a corresponding decline in their protein content.

In the panicles, which formed less than 12 per cent of the dry weight of the first growth and were absent from the aftermath, the protein was usually higher than in the leaves and varied independently of the latter. The blade formed from 53 to 81 per cent of the dry matter of the leaf and its protein content varied from 15.5 to 26.87 per cent, while that of the sheath varied from 6 to 13.2 per cent. The amount in the sheath was only from one-third to one-half as high as that in the blade and the two values rose and fell together.

A higher protein content was favored by early mowing, thin stands, and an increased supply of available nitrogen. In the aftermath the protein was less variable and in general much higher than in the first growth. Differences in the protein content of grasses of different strains, sown at the same time and harvested at the same stage of maturity, were at most very small compared with differences connected with the degree of maturity and supply of nitrogen.

The grass seeded broadcast gave a much lower yield of hay and a very much lower yield of protein than where it was sown in rows on adjacent similar land, similarly fertilized and the weeds and other grasses kept down until it became established. The yield and protein content of the hay from *Phalaris arundinacea* appears unusually

sensitive to the supply of available nitrogen in the soil, and the protein content is very much influenced by the stage of maturity and consequent proportion of culm and sheath.

LITERATURE CITED

- (1) ARENDT, R., and KNOP, W.
1860. GRASUNTERSUCHUNGEN. Landw. Vers. Sta. 2: 32-64.
- (2) ARNY, A. C., HANSEN, M. C., HODGSON, R. E., and NESOM, G. H.
1929. REED CANARY GRASS. Minn. Agr. Expt. Sta. Bul. 252, 19 p., illus.
- (3) BERSCH, W.
1912. HANDBUCH DER MOORKULTUR. FÜR LANDWIRTE, KULTURTECHNIKER UND STUDIERENDE. Aufl. 2, 310 p., illus. Wien and Leipzig.
- (4) CASSIDY, J., and O'BRINE, D.
1890. SOME COLORADO GRASSES AND THEIR CHEMICAL ANALYSIS. 1889. Colo. Agr. Expt. Sta. Bul. 12, 151 p., illus.
- (5) ERMERT.
1928. DER FUTTERWERT DES HEUS UND DIE WIRTSCHAFTLICHKEIT VON MOORMELIORATIONEN. Mitt. Ver. Förd. Moorkult. Deut. Reiche 46: 174-178.
- (6) ERWIN, R. E.
1894. REPORT OF CHEMICAL DIVISION. Utah Agr. Expt. Sta. Ann. Rpt. (1893) 4: 251-255.
- (7) FELDT, W.
1926. BERICHT ÜBER DIE TÄTIGKEIT DES MOORAMTES DES LANDWIRTSCHAFTSKAMMER FÜR DIE PROVINZ OSTPREUSSEN. Protokoll Sitzung Zent.-Moor-Komm. 85: 125-144, illus.
- (8) ———
1927. DIE ARBEITEN DES MOORAMTES DER LANDWIRTSCHAFTSKAMMER FÜR DIE PROVINZ OSTPREUSSEN IM JAHRE 1926. Protokoll Sitzung Zent.-Moor-Komm. 87: 89-108, illus.
- (9) FLINT, C. L.
1857. [BRIEF ACCOUNT OF THE NATURAL HISTORY OR DESCRIPTION OF ALL THE USEFUL GRASSES FOUND IN ... [MASSACHUSETTS] FIELDS AND PASTURES.] Sec. Mass. Bd. Agr. Ann. Rpt. 4: 5-284, illus.
- (10) GASPARIN, [A. E. P.] DE
1848. COURS D'AGRICULTURE. T. 4, 787 p. Paris.
- (11) HEUSER, O.
1927. GRUNDRISSE DER MOORKULTUR. 176 p., illus. Berlin and Leipzig.
- (12) HILLS, J. L.
1890. FEEDING TESTS OF MILCH COWS. Vt. Agr. Expt. Sta. Ann. Rpt. (1889) 3: [51]-86.
- (13) HOFFMANN, R.
1861. DIE PFLANZE. Jahresber. Fortschr. Agrikult. Chem. (1859/60) 2: [74]-183.
- (14) KELLGREN, A. G., and NILSON, L. F.
1893. UNDERSÖKNING OF SVENSKA FÖDER-ÖCH BETESVÄXTER. K. Landtbr. Akad. Handl. och Tidskr. 32: 1-32.
- (15) PIPER, C. V.
1915. FORAGE PLANTS AND THEIR CULTURE. 618 p., illus. New York.
- (16) ———
1925. CULTIVATED GRASSES OF SECONDARY IMPORTANCE. U. S. Dept. Agr. Bul. 1433, 43 p., illus.
- (17) RINDELL, A.
1908. ZUR KENNTNISS DES GLANZGRASES. Mitt. Ver. Förd. Moorkult. Deut. Reiche 26: 183-185.
- (18) SEELHORST, C. VON
1892. ACKER-UND WIESENBAU AUF MOORBODEN. 292 p., illus. Berlin.
- (19) SHEPARD, J. H., and WILLIAMS, T. A.
1894. NATIVE AND INTRODUCED FORAGE PLANTS. S. Dak. Agr. Expt. Sta. Bul. 40, 208 p., illus.
- (20) SPEER, R. L., WADE, C. M., and PATRICK, G. E.
1890. CULTIVATED AND WILD VARIETIES OF THE GRASSES IN IOWA. Iowa Agr. Expt. Sta. Bul. 11: 448-480.

-
- (21) STEBLER, F. G.
1898. DIE BESTEN STREUEPFLANZEN. 148 p., illus. Bern.
- (22) STORER, F. H.
1900. CHEMICAL COMPOSITION OF BLUE JOINT-GRASS (*CALAMAGROSTIS CANADENSIS*), AS CONTRASTED WITH THAT OF REED CANARY GRASS (*PHALARIS ARUNDINACEA*). Bul. Bussey Inst. 2: 130-136.
- (23) STRECKER, W.
1914. DIE KULTUR DER WIESEN, IHR WERT, IHRE VERBESSERUNG, DÜNGUNG UND PFLEGE. Aufl. 3, 462 p., illus. Berlin.
- (24) WEBER, C. A.
1928. DAS ROHRGLANGZGRAS UND DIE ROHRGLANGZGRASWIESEN NEBST ANDEREN WIESENARTEN DES NASSEN UND ZEITWEILIG ÜBERFLUTETEN BODENS. 48 p., illus. Berlin.
- (25) WERNER, H.
1889. HANDBUCH DES FUTTERBAUES. Aufl. 2, 467 p., illus. Berlin.

THE MORPHOLOGY AND BIOLOGY OF EULIMNERIA CRASSIFEMUR, AN IMPORTANT PARASITE OF THE EUROPEAN CORN BORER¹

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INTRODUCTION

Eulimneria crassifemur (Thomson) is an important parasite of the larva of *Pyrausta nubilalis* Hbn. in Europe and, with other parasites, has been studied by the staff of the European parasite laboratory at Auch and Hyères, France, since 1919. Shipments of this species to the United States have been made in varying quantities each year since that date. It is therefore deemed advisable to publish such information as is now possessed concerning its biology and habits in order to aid various scientific workers engaged in manipulating and colonizing this parasite.

SYSTEMATIC POSITION AND SYNONYMY

This parasite is a species of the genus *Eulimneria*, the tribe Campoplegini, subfamily Ophioninae, family Ichenumonidae, and order Hymenoptera. It was first described by Thomson (13, p. 1106)⁴ as a species of Holmgren's genus *Limneria*. This name was changed to *Limnerium* by Ashmead in 1900 (1). In 1907 the genus *Eulimneria* was erected by Schmiedeknecht (7) for the group of species including *crassifemur*. Szépligeti (12, p. 16), however, lists it under *Limnerium* in a fascicle of the *Genera Insectorum* published in 1911. Smits van Burgst (10, p. 144) considers this species identical with *Campoplex lineolatus* Bouché, a parasite of (*Tinea*) *Hyponomeuta evonymella* L., a European apple pest (Lepidoptera).

GEOGRAPHICAL DISTRIBUTION

Originally described from Sweden and cited by Dalla Torre (4, p. 93) in his *Catalogus Hymenopterorum* as occurring there, this species is listed in *Genera Insectorum* as occurring in Sweden and Germany. It occurs also in Belgium, France, Italy, Yugoslavia, Hungary, Switzerland, and Spain. In France the parasite has been found in the following localities: The southwestern plain, extending up into the valleys of the Pyrenees; the Rhône Valley around Valence (but not south of Avignon); the valley of the Main around Angers; the basin of the Seine around Paris; the region of Lille; the Rhine Valley around Strasbourg and Colmar; and the region of the Jura near Sellières. This species has never been taken at Hyères or at any other point in the French Mediterranean zone. In Belgium it occurs at Brussels and Antwerp. In Italy it has been taken at Pavia, Bergamo, and Piacenza in the Po Valley, as well as at Udine and Naples. In Spain it has been found in the Province of Galicia.

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² Resigned June 1, 1928.

³ The authors acknowledge their indebtedness to Mrs. W. R. Thompson for her assistance in the preparation of many of the illustrations.

⁴ Reference is made by number (*italic*) to "Literature cited," p. 344.

HOST RELATIONS

Eulimneria crassifemur has been reared by Schwangart (8, p. 555) as a parasite of the larvae of (*Conchylis*) *Clysia ambiguella* Hbn., the grapevine conchylis, and (*Eudemis*) *Polychrosis botrana* Schiff., the European grape berry moth, in Austria. Trägårdh (19, p. 420) reared it from *Lyda signata* Fab., the spruce sawfly, while Smits van Burgst (10, p. 144) reports it from *Evetria buoliana* Schiff., the European pine shoot moth. The writers are of the opinion that the *E. crassifemur* reared from *Pyrausta nubilalis* may not be identical with the species reared from the above-mentioned hosts.

Paillot (5, p. 190) has reared a species which he calls *Eulimneria crassifemur* from (*Neurotoma*) *Lyda nemoralis* L., the peach sawfly,

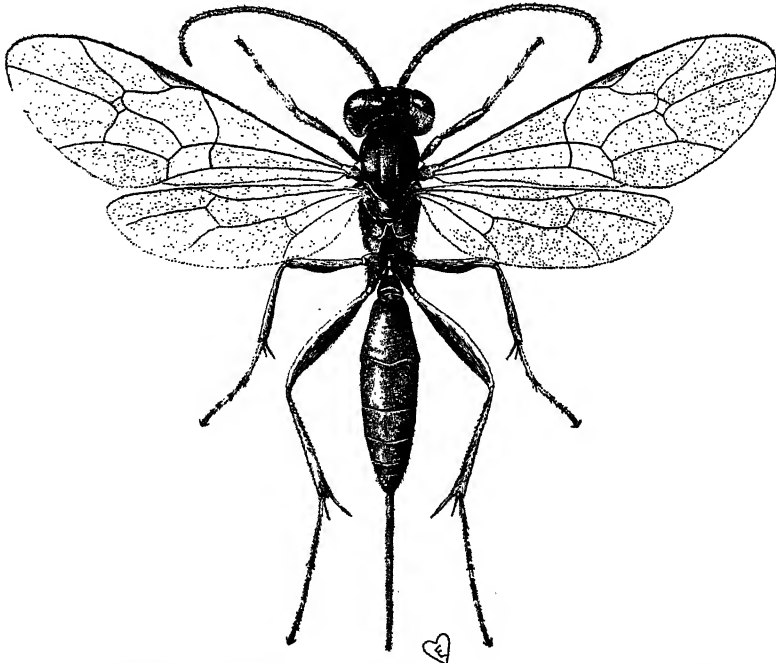


FIGURE 1.—*Eulimneria crassifemur*, adult female. The second and third abdominal segments appear considerably foreshortened in this drawing owing to the curvature of the body

but, according to this author, his species differs from the parasite of *Pyrausta nubilalis* in that it has a shorter ovipositor.

The writers of the present paper have reared *Eulimneria crassifemur* from *Pyrausta nubilalis*, collected from all the localities mentioned in Spain, France, Belgium, and Italy, and from south Wurttemberg in Germany, while K. W. Babcock and A. N. Vance have reared it in Hungary and Yugoslavia.

DESCRIPTION OF THE ADULT

Eulimneria crassifemur (fig. 1) is a black-bodied, red-legged, wasplike insect about one-half inch long, with a protruding ovipositor. It differs from the other campoplegines parasitic on *Py-*

rausta nubilalis in having an areolet in the forewings, no yellow or red on the body, middle and hind coxae and hind trochanters black, and tips of hind tibiae and hind tarsi black, but proximal extremities of first three tarsal joints dirty white. The ovipositor is about one-half the length of the abdomen. This species is more likely to be confused with *Diectes punctoria* Roman than with any other parasite of the corn borer, but it can be readily separated from that species by the presence of the areolet.

DESCRIPTION OF IMMATURE STAGES

EGG

The egg (fig. 2, A) averages 2.9 mm. long and 0.9 mm. wide. It is oblong ovate, slightly flattened on one side, and entirely without spines, sculpturing, or processes of any kind. When first deposited it is white, but within a few days it turns a dark brown and can then be distinguished through the skin of the host larva.

PRIMARY LARVA

The first-stage larva (fig. 3, A) is of the typical ichneumoniform type. Cylindrical, without spines, tubercles, or segmentary appendages, composed of a heavily chitinated head followed by 13 body segments; the last or tenth abdominal segment prolonged ventrally into a conical, pointed tail about one-half as long as the rest of the body.

Head thimble-shaped, slightly pointed anteriorly, and of a light brown color; dorsally from the posterior border two thinly chitinated grooves extend forward over three-fourths of the length of the head; laterally (fig. 2, H) with an irregular series of small circular sensorial organs; dorsally (fig. 2, D) one pair of large sensorial hairs; posteriorly and slightly above the lateral line another pair, while on the ventral portion there are four such pairs. Mouth parts distinctly delineated. The labrum with seven pairs of sensorial organs, the labial region with three, and the maxillary region with three. (Fig. 2, B.) Mandibles simple, strongly curved, and rather sharply pointed.

Body cylindrical, tapering but slightly posteriorly, segments about equal in length. The cuticle bears no tubercles or spines and no visible sensorial organs.

Digestive system well developed and the fore, middle, and hind intestines readily distinguished as well as the Malpighian tubes and the branched salivary glands, which extend the entire length of the mid intestine. Brain and ventral nerve cord prominent, the heart distinguishable by its more or less regular pulsations at intervals of three to four seconds. The fat body but slightly developed, and the only part of it that can be readily distinguished is in the tail (figs. 2, E and 3, B); small fibers which are probably hypodermal attach this lobe of the fat body to the hypodermal cells of the tail.

The tracheal system (fig. 3, B) is as follows: A trunk runs along each side of the body from the first thoracic to the ninth abdominal segment; these trunks are joined anteriorly by a transverse commissure which passes above the digestive tract in the first thoracic segment; posteriorly they are joined in a like manner by a ventral commissure in the anterior portion of the ninth abdominal segment. A secondary lateral trunk extends from the posterior margin of the first thoracic to the posterior margin of the third thoracic segment.

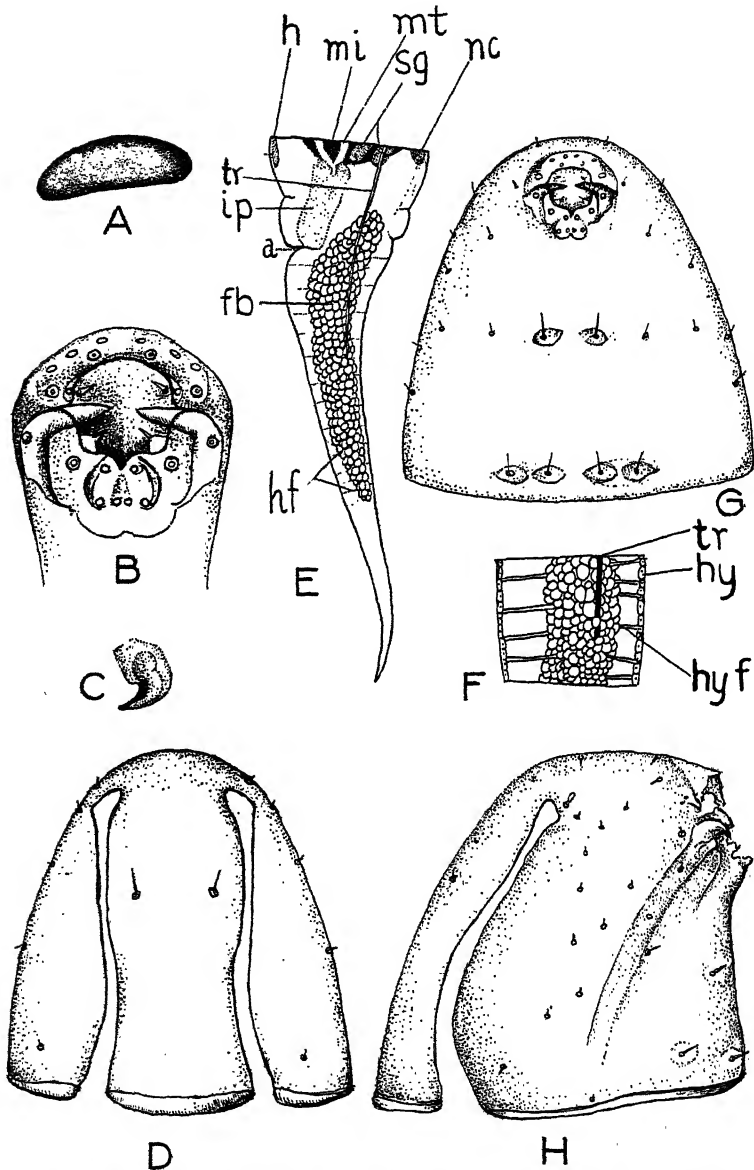


FIGURE 2.—*Eulimneria crassifemur*: A, egg, several days old; B to H, larval details; B, mouth parts of stage 1; C, mandible of stage 2; D, dorsal view of head skeleton of stage 1; E, caudal extremity of stage 1 showing fat body (fb), trachea (tr), hypodermal fibers (hf), nerve cord (nc), hind intestine (ip), midintestine (mi), heart (h), Malpighian tubes (sg), salivary glands (sa), and anus (a); F, portion of caudal extremity enlarged, section about middle of the fat body, showing trachea (tr) and its connection with the fat body, hypodermis (hy), and hypodermal fibers (hyf); G, ventral view of head of stage 1; H, lateral view of head skeleton of stage 1.

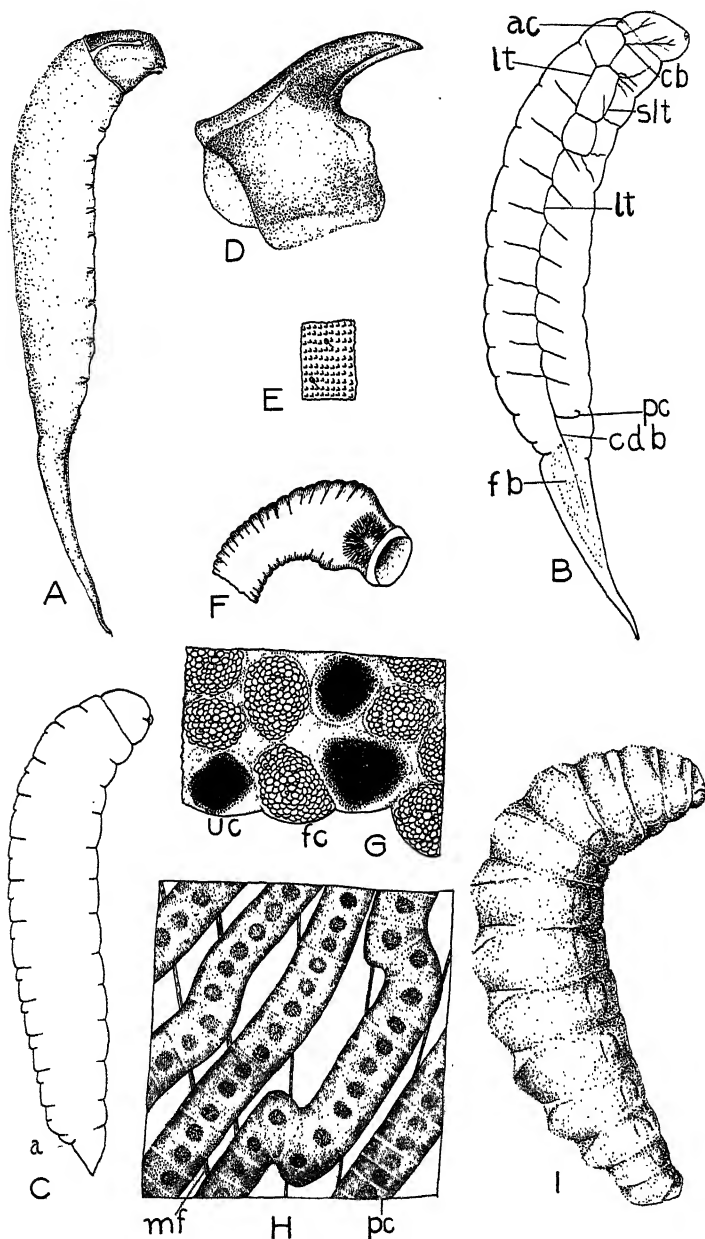


FIGURE 3.—Larval details of *Eulimneria crassifemur*: A, First-stage larva before feeding; B, first-stage larva after feeding, showing tracheal system, fat body (fb) in caudal process, anterior commissure (ac), posterior commissure (pc), lateral trunk (lt), secondary lateral trunk (slt), cephalic branch (cb), and caudal branch (cdb); C, side view of stage 2 larva, (a) anus; D, mandible of full-grown larva; E, portion of skin of full-grown larva showing microscopic tubercles which entirely cover the body and two of the sensorial papillae; F, spiracle of full-grown larva; G, portion of fat body near anterior end of stomach where the urate cells appear, seen by transmitted light, fat cells (fc), urate cells (uc); H, small portion of pericardial membrane showing pericardial cells (pc) arranged in irregular tubules; and dilator muscle fibers (mf); I, side view of full-grown larva.

This trunk is attached to the principal lateral trunk by three dorso-ventral commissures located in the first to third thoracic segments; this secondary lateral thoracic trunk, which Seurat appears to have been the first to notice, seems to occur throughout the Ichneumonidae and is probably characteristic of that group. The anterior transverse commissure gives off branches to the head, the main lateral trunk furnishes a dorsal and a ventral branch to each segment, the secondary lateral trunk furnishes ventral and lateral branches to the three thoracic segments, and a branch arising at the posterior end of the main lateral trunk continues into the tail, where it becomes lost among the fat cells which fill the lumen of that organ. The lumen of the tail is continuous with that of the body. There are no open spiracles.

The tail, which exists in various forms in a considerable variety of parasitic larvae (Ichneumonidae, Braconidae, Chalcididae), has been the subject of a good deal of rather fruitless speculation. Seurat (9, p. 90) considers it to be a locomotory organ, Timberlake (17, p. 85) and others to be a blood gill, Weissenberg (according to Tothill) believes it to be used for storing excretory substances.

Tothill (18, p. 71) considers the tail to be a respiratory organ. According to his description of the larva of *Campoplex pilosulus* Prov., it is anatomically a special sort of gill and is composed of a mass of small tracheal tubes each of which terminates in a large vesicle lying in the "wall" of the gill, which is thus lined with numerous large air cavities separated by narrow protoplasmic walls. The writers have identified without difficulty in the larva of *Eulimneria crassifemur* all the structures represented by Tothill in his figures of the larva of *C. pilosulus*. They are convinced, however, that Tothill's interpretation of his preparations is erroneous. The mass of material represented within the cavity of the tail in Tothill's Figure 55, and said by him to be a bundle of tracheids, is in reality simply a lobe of adipose tissue from which the fat globules have been dissolved out by the reagents employed, so that the appearance in section is similar to that of a mass of tubules. The so-called intracellular air spaces into which the tracheids are said to open in the wall of the tail are simply extensions of the body cavity incompletely separated by protoplasmic strands running from the hypodermis of the tail to the adipose tissue lying within it. The true structure of the tail is easily determined by the examination of living larvae, in which it can be seen that the only respiratory structures of the organ are two fine tracheae which pass backward into the lobe of adipose tissue; that the so-called intracellular air spaces contain no air, but are filled with liquid; and, finally, that the central mass described by Tothill is a lobe of the fat body. The tail is therefore not anatomically a tracheal gill. None of the hypotheses which have been advanced in regard to its function appear to the writers to be satisfactory, and they are inclined to doubt that this organ, in spite of its anatomical prominence in certain parasitic larvae, is physiologically of any particular importance.

SECONDARY LARVA

The secondary larva (fig. 3, C), as is generally the case in this type of hymenopterous larvae, differs from the primary larva principally by its large size, its soft and more hemispherical head, and its shrunken tail process. There are no open spiracles in this stage.

MATURE LARVA

Mature larva (fig. 3, I) 10 mm. long and 3 mm. wide, subcylindrical, tapering slightly anteriorly and posteriorly. General color a dirty white, with the snow-white urate cells showing through the skin. Cuticle with tiny tubercles thinly spaced, visible only under the microscope. Head hemispherical but slightly flattened dorsoventrally; with numerous sensorial organs around the mouth parts⁴ and on its upper surface a pair of brownish disks which correspond to the antennae. The various appendages of the buccal region distinctly represented. Each of the thoracic segments bears ventrally a pair of light-brown disks representing the legs, while the second and third segments bear a lateral pair of similar disks representing the wings. Each segment has a number of tiny hairlike sensoria, the number and arrangement of which are shown approximately in the diagram in Figure 4, C. The eighth and ninth abdominal segments each bear a pair of light-brown disks in the female larva, whereas in the male larva there is only a single pair located on the posterior edge of the ninth abdominal segment. These disks surround the pedicels of the histoblasts of the external reproductive organs. The posterior lobe, which represents the remains of the tail process, terminates in a small cuticular spike.

The tracheal system (fig. 5, A) is essentially the same as in the primary larva; the branches are, however, considerably more ramified and there are now nine pairs of open spiracles located on the first thoracic and the first eight abdominal segments.

The integumentary muscular system (fig. 4, A) consists of a longitudinal dorsolateral group (dl1-dl6), a ventrolateral group (vl1-vl6), a series of diagonal muscles extending anteriorly in a dorsoventral direction (a1-a4), a series of diagonal muscles extending anteriorly in a ventrodorsal direction (b1-b3), and a series of small dorsoventral muscles (t1-t5) of which four occur only in abdominal segments 1 to 9, inclusive. The diagonal muscles b1 and b2 and a1 do not traverse the entire length of the segment but are attached to the integument at a point somewhat behind the forward intersegmental line. It is this attachment which gives the double segmental appearance to the *Eulimneria* when seen in profile. In the ninth abdominal segment the muscle dl3 extends backward across the tenth segment and attaches to the rectum, and there seems to be a supplementary diagonal muscle (a5) in about the same position as a2 but external to the transverse muscle (t2-5). The integumental musculature is thus of a very simple type.

The cephalic musculature is composed principally of four groups, the first of which extends from the labrum to the wall of the head immediately above it, the second group extends in like manner from the esophagus upward to the chitin of the head, the third group extends from near the back of the head forward to the region around the mouth, while the fourth group extends from the periphery of the head near the posterior lateral border to the region of the labium. The labial and esophageal groups are active principally during the process of ingestion.

The fat body (fig. 3, G) consists principally of four more or less regular lobes surrounding the digestive tract, placed internally to the

⁴ The arrangement of these organs is constant and will, the writers are convinced, prove of considerable value for purposes of identification; it seems to be identical in *Eulimneria crassifemur* and *Diocles punctaria* Roman.

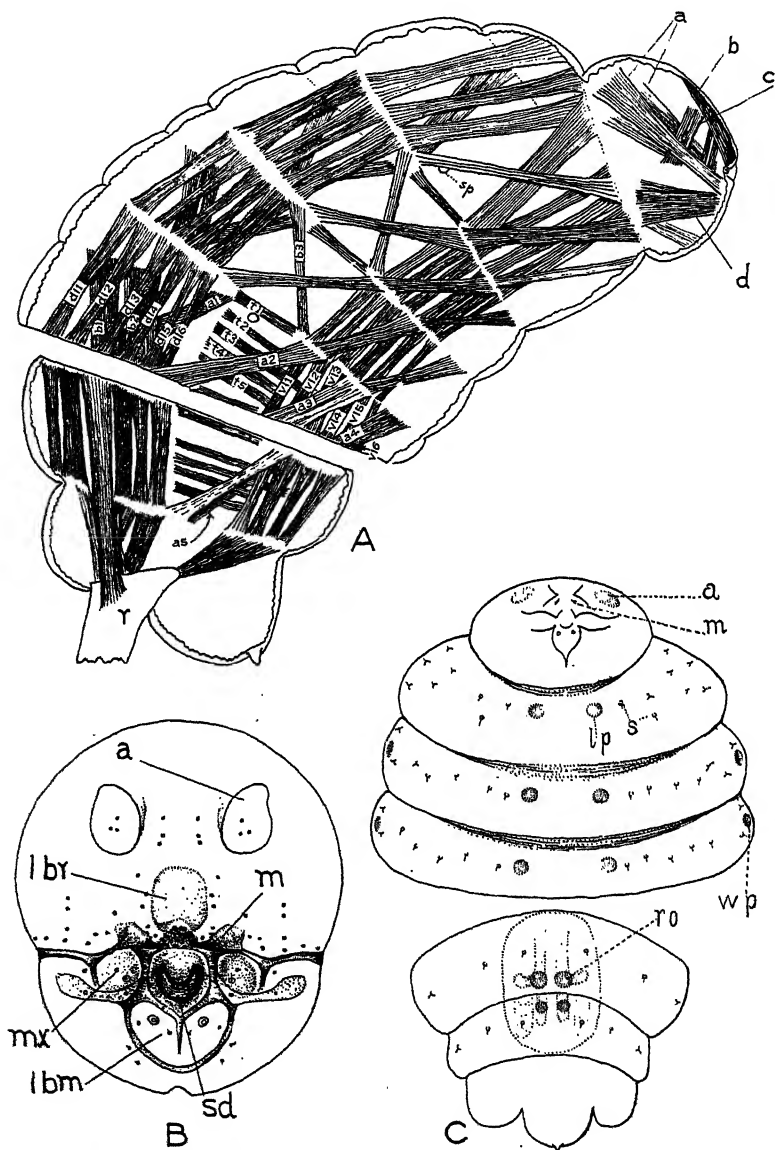


FIGURE 4.—Larval details of *Eulimneria crassifemur*: A, Integumentary muscles of full-grown larva (left side) showing the head, the three thoracic, and the first, ninth, and tenth abdominal segments, dorsolateral group (dl), ventrolateral group (vl), anterodorsovenral diagonal muscles (a), anteroventrodorsal diagonal muscles (b) dorsoventral transverse muscles (t), spiracle (sp), and rectum (r); B, head of full-grown larva (front view), antenna (a), labrum (lbr), mandible (m), maxilla (mx), labium (lbm), salivary duct (sd); C, drawing of head, thorax, and eighth to tenth abdominal segments (from below) of full-grown female larva to show external organs, sensoria, etc., leg pads (lp), wing pads (wp), reproductive organs (ro), sensoria (s), antenna (a), and mandible (m)

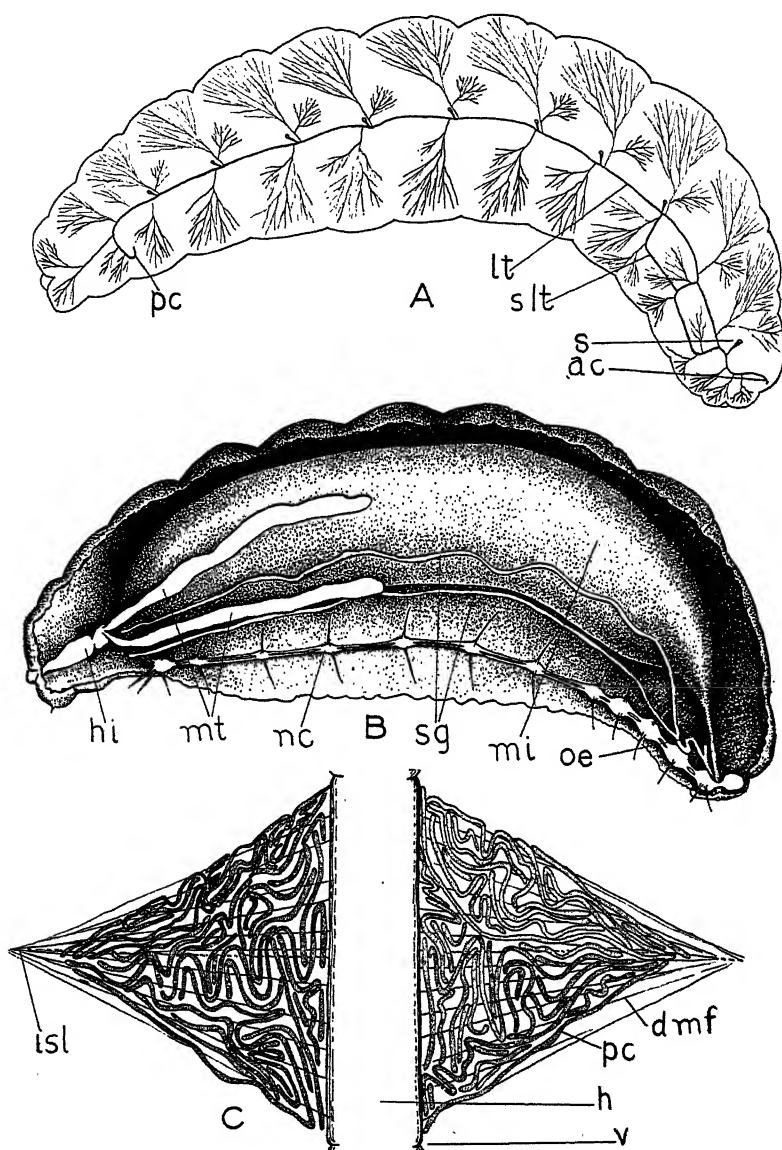


FIGURE 5.—Larval organs of *Eulimneria crassifemur*: A, Respiratory system of full-grown larva, spiracle (*s*), lateral trunk (*lt*), secondary lateral trunk (*s/lt*), anterior commissure (*ac*), posterior commissure (*pc*); B, internal organs, esophagus (*oe*), mid intestine or stomach (*mi*), hind intestine (*hi*), salivary gland (*sg*), Malpighian tubes (*mt*), nerve cord (*nc*); C, segment of heart showing pericardial cells (*pc*), dilator muscle fibers (*dmf*), valve (*v*), and intersegmental line (*isl*)

integumentary muscles, while in each segment between the skin and the integumentary muscles there is a thin layer of adipose tissue. Miscellaneous conglomerations of minor importance exist at several other points in the anterior and posterior ends of the larva. The fat body is grayish white and the cells are quite distinct under magnification; the lobes which surround the mid intestine are thickly interspersed with urate cells wherein the accumulated granules are seen as snow-white lumps distributed among the fat. It is remarked that these urate cells occur only in the fat which is in contact with the mid intestine; the anterior and posterior ends of these fat lobes do not bear any urate cells nor do the lobes external to the integumentary muscles or the miscellaneous lobes. Figure 3, G, shows the fat cells as seen by transmitted light in tissue taken from the anterior extremity of the urate cell region.

The digestive tract (fig. 5, B) consists of the short, almost straight esophagus, fore intestine, mid intestine, or enlarged stomach without a posterior opening, and the hind intestine. The hind intestine is composed of two enlarged portions and a narrow rectum. The enlarged portions are separated by a narrow constriction in which there is a valve. The salivary glands consist of four slender tubes extending along the entire length of the mid intestine, the two tubes of each side uniting in the thorax and opening into the mouth by a very short common duct. Tothill (18, p. 66, fig. 41) describes in *Campoplex pilosulus* a "cut-off" duct leading from the salivary gland duct to the esophagus. The writers have not observed any such communication between these ducts in *Eulimneria* and are inclined to doubt its existence.

The Malpighian tubes arise from the hind intestine and extend forward almost one-half the length of the stomach; they are four in number and are larger than the salivary glands.

The nerve cord (fig. 5, B, *nc*) consists of the supraesophageal and subesophageal ganglia and 11 additional paired ganglia, the last of which probably represents three paired ganglia united as it gives off three pairs of branches. Tothill (18) shows 13 distinct and widely separated pairs of ganglia behind the subesophageal ganglia in his figures of *Campoplex pilosulus*.

The heart or dorsal vessel (figs. 3, H, and 5, C) is a narrow straight tube with the usual chambers and valves as found in hymenopterous larvae. It originates in the ninth abdominal segment and extends forward to the brain. It is bordered on each side from the ninth to the second abdominal segments by fan-shaped series of pericardial cells (figs. 3, H, and 5, C) which lie ventrally to a series of fine muscular fibers attaching the heart walls to the integument. The heart beats more or less regularly every three to four seconds with occasional pauses. With each pulsation the brain moves slightly.

PREPUPA

The prepupa is very much like the larva except that it is less arched dorsally, slightly more pointed anteriorly, and somewhat more yellowish in color. The red eyes can be seen beneath the old larval skin as can the other imaginal disks.

COCOON

The winter cocoon (fig. 6) varies from light gray to almost black. It is oblong oval, of solid texture; the lighter colored specimens exhibit

a faint whitish ring around the middle, but in the dark specimens no such ring is visible. The summer cocoons, which have been found only in northern Italy, are often thin and whitish with a distinct, paler ring around the middle. The summer generation in that region spins a mixture of the thick and thin types of cocoons from both of which the adults emerge before the winter sets in.

BIOLOGY

HABITS OF THE ADULT

The adult, having shed the old pupal skin, chews an irregular hole about 2 mm. in diameter slightly to one side of the end of the cocoon and, passing through this, makes its way to the outside through the *Pyrausta* tunnel.

If an artificial opening is made near the anterior end of the cocoon it is not used; the adult chews the usual, and now quite unnecessary, orifice to one side of it through which it emerges.

The adult *Eulimneria crassifemur* is an active, nervous insect which is seldom motionless and is quickly attracted to the light. In the field it can be observed flying rapidly to and fro, pausing on cornflowers to examine with the antennae, or by thrust of the ovipositor, places where *Pyrausta* larvae are, or have been, feeding. In captivity, especially if confined within a small space, the adults make frantic efforts to escape, beating themselves violently against the sides of the cage. These efforts diminish

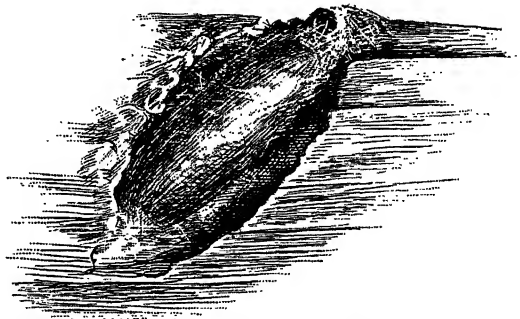


FIGURE 6.—Cocoon of *Eulimneria crassifemur* in tunnel in cornstalk

somewhat if the cage be made larger, and if the insects are placed in a cage high enough to admit a man standing they become somewhat more peaceful and fly around the cage here and there, lighting and crawling upon the walls, mostly toward the light. If sweetened water is given them, the females, and to a certain extent the males, are attracted to it, but when the latter become aware of the presence of a female they cease feeding to engage in mating.

In captivity both sexes of *Eulimneria* feed avidly on honey or sweetened water. It is almost certain that they do not as adults feed on the blood or body of the host larva as do so many chalcids, braconids, and some other ichneumonids.

The writers have no data concerning the length of life of this species in nature. Adults which emerged in the Hyères laboratory during the winter from cocoons formed in the preceding fall lived from 9 to 111 days. Table 1 gives the length of life of individuals kept at different temperatures and shows that the average longevity diminishes rapidly as the temperature rises.

TABLE 1.—Length of life of *Eulimneria crassifemur* adults at various temperatures, Hyères, France

Temperature	Length of life of various individuals	Average length of life for fed individuals
°C.	Days	Days
6.5	" 41, " 47, 65, 106, 111	94
10.0	58, 67	62.5
16.0	18, 41, 42, 59, 70, 77	51.2
23.0	" 33, 41	41
26.0	9, 32	20.5

* These individuals were not fed. All others were fed at more or less regular intervals.

Copulation may take place immediately after emergence, though in laboratory mating it is found to be a good practice to separate the sexes for from 24 to 48 hours, after which the mutual attraction seems to be more intense. In captivity this species does not mate readily; in small containers, such as glass tubes, lamp chimneys, or small screen-wire cages, the individuals will not mate, but fly about continually and knock themselves against the sides. For successful mating it is essential that the female should remain motionless during a period of preliminary "courtship" by the male. Smearing a few drops of honey or sugar water on the walls of the cage holds the females and allows the preliminary courtship to take place. If, after a period of isolation, the sexes are liberated in a large outdoor cage of screen wire copulation will readily take place. It seems that a moderate breeze blowing upon the parasites facilitates mating. The explanation of this is probably to be found in the fact that the females tend to cling more tightly to the screen in order not to be blown off, and the males are thus able to accomplish their preliminary maneuvers. These observations are substantiated by the behavior of the individuals after liberation in the field. As soon as the "liberating box" is opened the adults begin crawling out on the top and to this they cling for some little time. In a few minutes the top and sides of the liberating cage are covered with mating pairs.

In mating, the male approaches the female and begins fanning her by buzzing his wings rapidly at more or less regular intervals, at the same time raising somewhat the forward part of his body and moving around the female from side to side or in front, gradually approaching her; these movements require from one to two minutes or more for their completion; the male then mounts swiftly upon the back of the female and copulation takes place. Sexual contact continues for about three minutes.

The male is attracted to the female whether or not she has been already fertilized. Males often try, sometimes successfully, to copulate a second time with a fertilized female. A male seems to be able to fertilize several females before his sexual activity begins to diminish.

The exact length of time normally required between emergence and the beginning of oviposition has never been determined. Females have, however, been observed to attempt oviposition within 24 hours after issuing, even though unmated. Dissections of newly emerged females show that there are about 100 fully formed eggs in the oviducts and ovarian chambers.

When fragments of corn plants are placed in a cage with the female she examines them with the antennae, and if *Pyrausta* larvae have been feeding in the material in question she begins to explore it with the ovipositor, bringing the abdomen forward under the thorax and thrusting the dart here and there among the leaves and flowers. At the beginning of each exploration the sheaths guide the ovipositor to a forward position, whereupon they release their hold and resume their normal position. The exploration is continued until the ovipositor comes in contact with a larva; the dart is then rapidly inserted and an egg deposited. Within from 5 to 10 seconds the dart is withdrawn and the search for caterpillars resumed. The behavior of the female with respect to the hidden larvae of the host strongly suggests that sensorial organs located at the tip of the ovipositor play an important part in the identification of the host.

If a small larva is presented to a *Eulimneria* female without the protective covering of cornflowers, webs, etc., she will immediately rush upon it, give it a quick thrust with her ovipositor, and be off. The ovipositor is inserted at almost any point of the body, though in the laying cages, owing to the fact that the larvae attempt to crawl away from the direction of the attack, the thrust is often received in the posterior extremity. Sometimes the female deposits only one egg in the host, but often she deposits several, especially if she has been long denied access to hosts.

From 3 to 7 eggs are commonly found in caterpillars collected in the field, as many as 21 having been counted in a single host larva. From one lot of 72 caterpillars parasitized by *Eulimneria*, 167 eggs of this ichneumonid were obtained by dissection. The concentration of eggs in individual hosts is much too great to be due to chance and can only be attributed to a habit peculiar to this particular parasite.

The sting appears to produce momentary pain, as the host larva squirms and wriggles violently under the thrust, but this soon passes and the victim apparently suffers no permanent ill effect from the sting itself, though if the host is very small—in the first or second stage for example—the sting seems to paralyze, and in some cases the larva does not recover. Even a healthy larva in the third or fourth stage which has been stung repeatedly within a short time will become paralyzed and die.

The *Eulimneria* female oviposits best in *Pyrausta nubilalis* larvae of stages 3 and 4; she will deposit eggs in stages 2 and 5, though the small larva is sometimes overlooked and the skin of the fifth-stage larva is too thick and tough readily to admit the ovipositor, so the eggs are often found lodged in the skin half inside and half out. In addition, the females seem to have a general reluctance to attempt oviposition in larvae of stage 5.

Larvae of other species seem to attract them but little. In one instance healthy ovipositing females were supplied, in the midst of a series of young *Pyrausta nubilalis* larvae, with small larvae of the lepidopteron *Grapholitha rufillana* Staint., which breeds in the heads of wild carrots (*Daucus carota*). The females rushed at the *Grapholitha* larvae as they had been in the habit of doing with the *Pyrausta nubilalis* larvae, but just at the moment of oviposition they seemed to perceive something wrong and passed rapidly on to another object, some individuals giving a half-hearted thrust, others refraining

completely. A few eggs were deposited in these larvae but none of the resulting parasites came through to maturity.

HATCHING AND GROWTH OF LARVA

In a very interesting paper published in 1926, Cushman (3, pp. 42-43) deals with the various types of parasitism among the Ichneumonidae with particular reference to larval form and habits. This species on account of its habits and larval anatomy falls into Cushman's third type of internal parasites, along with *Limnerium validum* Cress., *Campoplex pilosulus*, and other campoplegines.

The eggs float freely in the body cavity of the host, but by reason of the peristaltic movements of the intestine and the circulation of the blood they soon come to be lodged in the extreme posterior end of the larva among the Malpighian tubes.

The eggshell splits open at one end and allows the young larva to squirm its way out. The shell remains with other fragments of débris packed in the posterior part of the host's body cavity and can be seen even when the Eulimneria larva has obtained its full growth. It is usually surrounded by a mass of phagocytes.

When several eggs are laid in one host larva only one Eulimneria survives to maturity. Their remains indicate that the surplus larvae are killed off by the first Eulimneria to hatch, or at all events by one of the earlier ones; it is known positively that the individuals which succumb all die very soon after hatching as they are often found dead only partly emerged from the egg and always in the first stage. In one instance a partially emerged larva dead with the mandibles of another first-stage larva fixed firmly in its body was observed. In this case the attacking larva also was dead, as well as a number of other first-stage larvae of Eulimneria in this same host.

It seems unlikely, however, that the death of the supernumerary individuals is in general due to a series of combats between the larvae. It is more probable, as Spencer (11, p. 134) has suggested in the similar case of Aphidius, that at a certain moment, soon after the hatching, the larvae begin to pour into the blood a cytolytic enzyme which affects the tissues of the host, and those of the younger larvae of Eulimneria itself.

The presence of a live Eulimneria larva in the body cavity of the host caterpillar does not seem to affect the health of the latter, at least during the parasite's first stage, for during this period the parasite is very small and seems to procure its nourishment without attacking the organs of its host. As the parasite larva grows it molts at least twice, and by the time it reaches the third stage it occupies three-fourths of the body cavity of its host. At about this stage the Eulimneria larva absorbs the material of the host's organs and when full fed it pierces a small hole in the skin of the now dead corn borer, pushes its way out, and spins its cocoon, to which the old host skin usually clings by a few threads. If the parasite larva be removed from the Pyrausta tunnel and placed upon a flat surface it is unable to spin a cocoon but scatters its silk over the surface. This is also true of a great many of the spinning larvae.

The cocoon may be opened toward the end of the pupal period without necessarily causing any ill effects to the larva, but if the larva is removed shortly after it has finished spinning it usually dries up and dies as do larvae which have been unable to form their cocoons.

When the winter is past, and the rise in temperature determines the resumption of activity, the larva expels its meconium, sheds its skin, and transforms into the typical white hymenopterous pupa. This pupa gradually darkens until the completely formed adult breaks out of the pupal skin and issues as already described.

HIBERNATION

In a paper published several years ago on the phenomena of hibernation in the Muscidae, E. Roubaud (6, p. 469) classified insects into two categories, the homodynamic, which included species capable under favorable conditions of uninterrupted development for an indefinite number of generations, and the heterodynamic, which included species in which an obligatory resting period occurs in some stage or other at periodical intervals in the succession of generations. Hibernation or aestivation are in the homodynamous species determined purely by external conditions such as low temperature or dryness, while in the heterodynamic forms they are determined essentially by the physiological condition of the organism.

Eulimneria crassifemur is to some extent intermediate between these two types. The prepupal and pupal period of the summer generation is completed in from about 15 to 20 days. In the cocoons of the winter generation this phase may require four or five months. The recurrence of the diapause is not, however, independent of environmental conditions, for in the 1-generation areas it occurs in every generation, while in the 2-generation areas it occurs only once every two generations and only in the larvae which spin cocoons in the fall. Furthermore, unlike certain species of the heterodynamic group described by Roubaud, *E. crassifemur* is susceptible, even during the diapause, to the influence of high temperature. Thus a lot of cocoons collected in southwestern France on January 1, 1927, and kept in cold storage until February 10 were on that date divided into two lots, one of which was kept at 18° C. and the other at 27° C. The results of this experiment were that adults emerged from those kept at 27° C. in 11 days, whereas those kept at 18° C. did not produce adults until the twenty-second day. The exposure to high temperature thus produced a well-marked acceleration of development.

As the season advances, the time required for the completion of the pupal stage at a given temperature gradually decreases, proving that under field conditions the hibernating individuals are slowly developing. This is shown clearly in Table 2, which gives the results obtained by the incubation at 33.5° C. of cocoons collected at Auch, France, on a series of dates from December 29, 1924, to February 2, 1925. It will be noted that the number of days from the beginning of the experiment (January 5) to emergence increases as the date of collection advances, the reason for this being that development went on less rapidly in the specimens which remained in the field. The specimens placed at 33.5° C. on January 5 completed their development in only 10 days less than those taken from the field on February 2.

TABLE 2.—*Accelerated emergence of adults of Eulimneria crassifemur from cocoons placed at a temperature of 33.5° C. at different periods of natural development at Hyères, France*

Date of collection	Date placed in incubator ^a	Number of days from placing in incubator to emergence	Day of emergence counted from beginning of experiment on Jan. 5
Dec. 29, 1924	Jan. 5, 1925	33	33
Jan. 1, 1925	Jan. 7, 1925	31	33
Jan. 6, 1925	Jan. 13, 1925	26	34
Jan. 12, 1925	Jan. 19, 1925	14	28
Jan. 20, 1925	Jan. 27, 1925	13	35
Jan. 29, 1925	Feb. 5, 1925	9	40
Feb. 2, 1925	Feb. 9, 1925	8	43

^a During the interval between the date of collection at Auch and the beginning of the incubation at Hyères the cocoons were in transit and at a temperature probably slightly higher than in the field.

SEASONAL HISTORY

Eulimneria crassifemur occurs over the range of *Pyrausta nubilalis* in the 1-generation and "transitional" zones and in certain of the 2-generation zones of the latter. Throughout these zones the seasonal history of *Eulimneria* coincides more or less with the seasonal history of *P. nubilalis* except for the fact that in the transitional zones the parasite usually has only one generation with no partial second, whereas *P. nubilalis* may have a partial second even in unfavorable seasons. If the season is favorable, causing the host to pass through two full generations, the *Eulimneria* may have a partial or entire second generation. Such a partial second generation occurred at Auch, France, in 1920.

The seasonal history of *Eulimneria* for the various zones in which it has been studied (fig. 7) may be summarized as follows:

AQUITANIAN ZONE (SOUTHWESTERN FRANCE).—In this zone there is usually one generation of *Pyrausta nubilalis* and one of *Eulimneria*. The majority of the adults of *Eulimneria* emerge between March 15 and April 15, the heaviest emergence being about April 1. Some few individuals emerge earlier or later,⁶ the earliest record being February 9 and the latest May 26 (though one white nymph was found in its cocoon on June 6).

The writers believe that these adults wait about until the young *Pyrausta nubilalis* appear, about the 1st of August, and oviposit in them. (A spring generation in some intermediate host is possible, though, in the writers' opinion, unlikely.) The resulting parasite larvae reach maturity by the end of September or the middle of October, spin their cocoons, and remain over winter in the dried cornstalks as fully developed larvae, transforming into nymphs and adults the following spring.

If the season be very early and warm, the spring emergence of the host is somewhat advanced, as is also that of the parasite to a certain extent. This permits an entire or partial 2-generation cycle. In this case the earliest larvae of *Pyrausta nubilalis* emerge during the first half of July and are then parasitized by *Eulimneria*. The resulting

⁶ This seems to be an important point in the life history of *Eulimneria*. The writers believe that only the late-emerging individuals can live long enough to encounter the earliest host larvae. This matter will be discussed more fully later in this paper (p. 339).

generation of the parasite completes its development and emerges during the latter half of August, at which time, or shortly thereafter, young larvae of *nubilalis* of the second generation are present in the tips of the ears, at the bases of the greener leaves, and in fresher parts of the corn plant. The parasites oviposit immediately in these young caterpillars, and the cocoons of this generation are all spun by the 1st of November. The winter is then passed in the cocoon as in the case of the 1-generation cycle.

MEDITERRANEAN (NORTH) ZONE (RHONE VALLEY AROUND VALENCE).—In this region there is regularly only one generation of *Pyrausta nubilalis* with sometimes a small partial second generation amounting to 10 or 15 per cent of the total. With *Eulimneria*, however, there is only one generation so far as is known. The *Eulimneria*

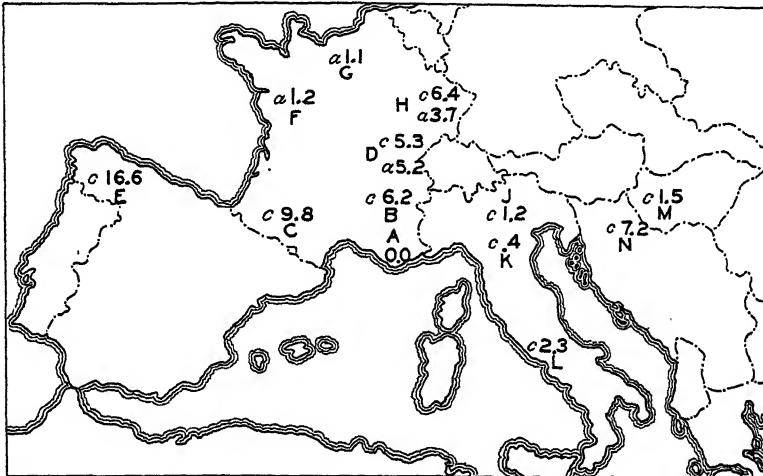


FIGURE 7.—Map showing the zones in Europe in which *Eulimneria crassifemur* has been studied: A, Mediterranean (south); B, Mediterranean (north); C, Aquitanian; D, Rhodanian; E, Galician; F, Armorican; G, Sequanian; H, Vosgian; J, Padovian (north); K, Padovian (south); L, Campanian; M, Hungarian; N, Yugoslavian. The figures represent the average percentage of parasitism of *Pyrausta nubilalis*. a, in *Artemisia*; c, in corn

cocoons from the first-generation hosts are formed in August, but no adults issue from them until the following spring.

SEQUANIAN ZONE (PARIS, LILLE).—In this zone there is only one generation of host and parasite. The adults issue at about the same time as, or but slightly later than, they do in the Auch (Aquitanian) region, and the eggs are laid in late July and early August, at which time larvae of *nubilalis* are available. The development of these larvae proceeds more slowly (as does that of the host) than in either the Aquitanian zone or in that of the Rhone Valley, the cocoons being formed during the latter part of September and early October. The plant in which *P. nubilalis* develops in this zone is *Artemisia vulgaris*.

ARMORICAN ZONE.—In the Armorican zone the seasonal history is practically identical with that of the Sequanian.

VOSGIAN ZONE (COLMAR, STRASBOURG).—In the Vosgian zone, so far as known, the seasonal history of *Eulimneria* is the same as at Paris (Sequanian zone). Both the host and parasite may occur,

however, in corn as well as in *Artemisia*. Young *Eulimneria* of the first and second stages have been found near Strasbourg at the end of August and in early September.

RHODANIAN ZONE (JURA).—In this zone there is but one generation of host and parasite. The seasonal history here differs from that of the Rhône Valley zone (Valence) by the fact that oviposition is somewhat later, development slower, and the cocoons formed toward the end of September and during October.

DANUBIAN ZONE (HUNGARY, YUGOSLAVIA).—This is also a 1-generation zone, resembling the Sequanian, the Rhodanian, and the Vosgian zones in that the larvae of both host and parasite develop very slowly. Young host larvae are present from the middle of July on to September, while the *Eulimneria* eggs are laid during the latter part of July and early August, the earlier stages (eggs and first-stage larvae) being found in the host as early as August 10 or 12 and as late as September. The cocoons are spun during the latter part of October and the early part of November. In years in which the season is advanced the development may be somewhat earlier, as in 1927 eggs and young larvae of *Eulimneria* were found by A. M. Vance early in July.

PADOVIAN ZONE (PO VALLEY, BERGAMO, PIACENZA, PAVIA, ETC.).—In this zone *Eulimneria crassifemur* has two distinct generations, corresponding more or less with the two host generations. Eggs of the first generation are laid in early July while the cocoons, which may be of either the thick or thin type (see p. 330), are formed during the latter part of July and the early part of August. The adults issue from August 15 to 31 and deposit eggs of the second generation. The overwintering cocoons are formed during the latter part of October and early November and the adults issue the following spring during May and even until as late as June 5.

CAMPANIAN ZONE (NAPLES).—The data for this region are somewhat meager, but it is known that there are at least two generations. Adults issue in the spring from February to April and perhaps later. As *Eulimneria* does not exist at Hyères on the French Riviera, the Mediterranean (south) zone, where the climate closely resembles that of Naples, the writers are led to believe that at Naples the *Eulimneria* adults migrate down from the inland valleys where a milder summer prevails. Cocoons of the first generation have been collected late in July and early in August.

The seasonal history of *Eulimneria crassifemur* is thus approximately as follows: In the typical 1-generation zones (Sequanian, Armoricain, Vosgian, Rhodanian, and Danubian) the adults issue in the spring from late February until late May, with the maximum emergence about April 15, and lay their eggs in the earliest host larvae during a period which may commence in early July and extend on until as late in the season as early September. During this time the progeny develop slowly, and the cocoons are formed in October and early November.

In the 2-generation zones (Padovian and Campanian) the adults issue in the spring at about the same time as in the 1-generation zones, laying, in late June and early July, eggs which develop more rapidly, producing adults in August which oviposit for the second generation. The cocoons of these are formed toward the end of October and pass through the winter.

In the transformation zones (Aquitanian and Rhône Valley) the oviposition may occur at the same time as in the single-generation

zones, and development may proceed, according to the temperature, (1) slowly or (2) rapidly. If it proceeds slowly there will be but one generation, the cocoons being formed in late September, October, and early November; if it proceeds rapidly cocoons may be formed and adults issue about the middle of August, in which case there will be two generations. If the development is more rapid than in (1) and less rapid than in (2), cocoons may be formed in August, and instead of giving adults at once they may go over the winter, furnishing adults the following spring. This latter condition has been encountered in the Rhône Valley (Mediterranean, north) zone but never in the Aquitanian.

GEOGRAPHICAL DISTRIBUTION IN RELATION TO CLIMATE

The study of *Eulimneria crassifemur* in Europe has led to the conclusion that the presence or absence of this species in a given region depends more upon temperature than upon any other meteorological factor.

As has already been shown (Table 2), the hibernating individuals, though they are more refractory to external influences than those of the summer generation, are nevertheless affected by a rise in temperature, which produces a definite acceleration of development.

The hibernating larvae of *Pyrausta nubilalis* are, however, much less responsive to variations in external conditions. Exposure to high temperature during the diapause (during the months of January and February, for example) produces simply the desiccation and death of the majority of the larvae and determines in those which survive a relatively slight acceleration of development. After the larvae have emerged from the diapause, variations in temperature have, of course, the same effect as upon any homodynamous insect.

The result of this difference between *Eulimneria* and *Pyrausta* is that rises in temperature during the hibernation period, i. e., warm winters, determine an asynchronism in the seasonal histories of the parasite and its host. In a warm winter the parasites will thus emerge and die long before any larvae of the host are available.

In regions where the accumulated temperatures during the hibernation period are sufficient to produce emergence early in the winter *Eulimneria crassifemur* is likely to be uncommon or absent unless alternate hosts can be utilized.

In the southern Mediterranean zone the winters are mild and warm days sufficiently frequent to cause a very early emergence (late October or early November) of *Eulimneria*. This climatic feature, coupled with the fact that few lepidopterous larvae that would be suitable as hosts exist in the field at this time, is taken to be the reason for the complete absence of the parasite in this zone.

The occasional appearance of *Eulimneria crassifemur* in Naples, where the climate is essentially similar to that of the southern Mediterranean zone, may be explained by an annual migration of the *Eulimneria* from the higher valleys behind Naples down toward the lowlands. At all events these valleys with their more severe winter would serve as a constant source of supply for the Campanian zone, whereas the southern Mediterranean zone is entirely cut off from any such districts by mountains, waste lands, and vineyards from which *Pyrausta nubilalis* is practically absent.

This hypothesis does not, of course, explain the variations in the apparent effectiveness (i. e., relative abundance and percentage of parasitism) of *Eulimneria* in the other zones where it is found. Many other factors would have to be considered before the causes of these variations would become clear. The reaction to temperature of the hibernating individuals in regions where the species has a 1-generation seasonal history may be quantitatively different from that of the hibernating individuals of the 2-generation zones. The attraction of the adult parasite to larvae in weeds such as *Artemisia vulgaris* may be less intense than toward larvae feeding in corn. In certain regions the species may have other hosts to which a considerable proportion of the population is attracted in every generation. The question of the abundance of this species in the various parts of its habitat is thus one of extreme complexity and probably has no simple solution applicable to all conditions.

EULIMNERIA CRASSIFEMUR AS A FACTOR IN THE CONTROL OF PYRAUSTA NUBILALIS IN EUROPE

Eulimneria crassifemur is one of the most widely distributed of the parasites of *Pyrausta nubilalis* in Europe and is constantly present in all of the zones except the Mediterranean (south). Table 3 shows its relative importance as a whole in the various zones and should be consulted in connection with the map (fig. 7).

TABLE 3.—Average and maximum parasitism by *Eulimneria crassifemur* in Europe during the period of investigations (the maximum parasitism was always by the first generation)

Zone	Average parasitism	Maximum parasitism	Host plant	Year in which maximum parasitism occurred
	<i>Per cent</i>	<i>Per cent</i>		
Galician *.....	16.60	16.60	Corn.....	1925
Aquitanian.....	9.87	27.90	do.....	1920
Yugoslavian.....	7.20	13.20	do.....	1926
Voglian *.....	6.40	6.40	do.....	1925
Mediterranean (north).....	6.20	6.20	do.....	1925
Rhodanian.....	5.35	5.70	do.....	1924
Do.....	5.20	9.00	Artemisia.....	1925
Voglian *.....	3.70	3.70	do.....	1925
Campanian.....	2.30	2.60	Corn.....	1924
Hungarian.....	1.58	3.30	do.....	1926
Padovian (north).....	1.24	3.00	do.....	1924
Armorican *.....	1.20	1.20	Artemisia.....	1925
Sequanian.....	1.10	3.50	do.....	1925
Padovian (south).....	.40	.40	Corn.....	1925
Mediterranean (south).....	00	00

* Only 1 year's observation available.

Table 4 shows in detail the percentage of parasitism of *Pyrausta* by *Eulimneria crassifemur*, by year and by generation, observed in corn and in *Artemisia* in the various zones during the years in which it has been studied. It should be pointed out that while the figure for the average parasitism in the Galician zone is higher than in any other region this figure is based upon a single year's observation and is likely to be misleading for the reason that *Pyrausta nubilalis* is extremely scarce in this zone. In reality *Eulimneria* is much more abundant in the Aquitanian zone than in any other region studied.

TABLE 4.—The percentage of parasitism of *Pyrausta* by *Eulimneria crassifemur*, by years and generations, as observed in corn and *Artemisia* in the various zones of Europe, 1919–1927

Year and generation	Mediterranean (north)		Aquitainian	Rhodanian		Galician	Armorian	Sequannian	Vosgian		Padovian (north)	Padovian (south)	Campanian	Hungarian	Yugoslavian
	Corn	Corn		Corn	Artemisia				Corn	Artemisia					
1919: Generation 1															
Generation 2		6.90													
1920: Generation 1															
Generation 2		27.90 9.90													
1921: Generation 1															
Generation 2		6.00													
1922: Generation 1								1.10							
Generation 2		3.50													
1923: Generation 1								1.00							
Generation 2															
1924: Generation 1								1.00							
Generation 2		4.30		5.70	1.07						3.00 .90		2.60		1.50
1925: Generation 1															
Generation 2	6.20	2.90		5.00	9.00	16.60	1.20	3.50	6.40	3.70	2.50	0.40	2.50	1.00	1.50
1926: Generation 1															
Generation 2		3.37					.70	.34			.50			3.30	13.20
1927: Generation 1															
Generation 2		2.40					.43	.84			3.12 .01				

LIMITING FACTORS IN THE EFFECTIVENESS OF THE PARASITE

A parasite having an effective reproductive rate which is equal to or greater than that of its host, and which increases at the expense of the latter from generation to generation, must eventually come to equal it in numbers and almost exterminate it.

A parasite having an effective reproductive rate equal to that of the host, and whose population at the beginning of the period considered is numerically equal to one-fourth of the host population, should bring about the practical extermination of the host in four generations (Thompson, 14, 15, 16).

In the first generation of *Pyrausta nubilalis* in 1920 in south-western France the population of *Eulimneria* larvae was, according to estimates based on 20,000 corn-borer larvae actually examined, more than one-fourth that of the host. If the *Eulimneria* reproduces at the same rate as the corn borer, it should, in the third generation following, have increased to the point where it would parasitize practically 100 per cent of the host.

What actually occurred was that the proportion of caterpillars parasitized by *Eulimneria* fell in the succeeding generations and has not, as far as known, been above 10 per cent in the seven years following 1920. During this time no marked increase in the population of *Pyrausta* has occurred. The writers are therefore obliged to conclude that *Eulimneria* does not reproduce even as rapidly as *Pyrausta*, but in fact much less rapidly.

Furthermore, as the data in Table 4 show, in no region where this insect has been studied has there been any real indication that *Eulimneria* is increasing at the expense of *Pyrausta*.

As the parasitic habit constitutes a distinct advantage for *Eulimneria* (since every *Eulimneria* emerging means at least one less *Pyrausta*), it follows that the survival of *Eulimneria* as a parasite of *Pyrausta* must be very seriously inhibited by limiting factors.

An important limiting factor is probably the low reproductive rate of *Eulimneria crassifemur* as compared to *Pyrausta*. The writers have as yet no accurate data as to the number of eggs ordinarily deposited by *E. crassifemur*, but dissections of females lead them to believe that a potential total of 500 eggs is present. It is certain, however, that, as is usual among insects, a large proportion of these eggs are not deposited.

Paillot (5, p. 191) estimates that the form studied by him contained in its ovaries not less than 300 eggs but states that the number of eggs actually deposited never attains this total, for the female succumbs before her ovaries are exhausted. The average number of eggs actually deposited by *Pyrausta nubilalis* seems, however, to be well over 300 according to Caffrey and Worthley (2, p. 108).

The wasteful distribution of eggs by *Eulimneria crassifemur* is also unfavorable to the species. The results so far obtained indicate that probably one-half the eggs deposited by the females are placed in caterpillars already containing at least one egg. As has been seen, only a single *Eulimneria* larva survives in such cases and in many instances all die.

The *Eulimneria* female possesses a moderately long ovipositor and is able with the aid of this organ to discover larvae of the corn borer concealed within the various parts of the corn plant. Nevertheless

the position of the caterpillars must make them in many cases quite inaccessible to the parasite. The proportion of larvae thus escaping attack is usually greater in the second generation than in the first, for in the first generation the larvae or their castings are more or less apparent in the young flowers, whereas in the second generation they are hidden away in the stalk or lie deep between the stalk and leaf base or have burrowed far into the midrib of the leaf, or into the ears where it is impossible for the *Eulimneria* adults to find them. As a matter of fact, as the data tabulated show, the parasitism by this species in the caterpillars of the second generation is normally much lower than in the first generation.

Secondary parasites, among which are *Pezomachus* spp., *Dibrachys* sp., and *Melittobia acasta* Walk., have been reared from *Eulimneria* cocoons, but these constitute a negligible limiting factor.

Finally, the susceptibility to high temperatures during the winter months, causing in many years the premature emergence of the adult parasites, must, in many regions, greatly reduce the effectiveness of the species.

A compensating factor is the late burning of cornstalks, a practice common in the southwestern corn region of France. If the stalks are burned early with both the parasite and the host still in them, both suffer the same relative diminution, but if they are burned after the parasite emerges and before the corn borer emerges, the relative abundance of the parasite would be decidedly augmented.

Other limiting factors, still unknown to entomologists, probably operate with greater or less intensity in certain seasons and are responsible for certain fluctuations that have been observed. There can, however, be little doubt that those already mentioned suffice to limit very effectively the work of *Eulimneria crassifemur* as a parasite of *Pyrausta nubilalis*, so that, though the parasite certainly plays a definite part in the control of *Pyrausta* over the greater part of its range, it is incapable alone of reducing an outbreak or of producing a notable decrease in the damage resulting from the work of this pest.

SUMMARY

Eulimneria crassifemur Thomson (Hymenoptera, Ichneumonidae) is an important parasite of the European corn borer in Europe, where distribution records have been obtained from Spain, France, Belgium, Germany, Sweden, Switzerland, Italy, and the central European plains.

The adult is a black-bodied, red-legged, wasplike insect about one-half inch long. The egg is simple, oblong-ovate, white when first deposited but turning black after several days. The first-stage larva is slender, segmented, possessing a rather heavily chitinated brown head with strong hooked mandibles and a long pointed caudal segment; it has well-developed internal organs, tracheal trunks and branches but no open spiracles. The tail appendage is not a tracheal gill, as some have supposed. The mature larva is dirty white, of the typical "hymenopteriform" type with well-differentiated mouth parts, simple mandibles, and nine pairs of open spiracles. The winter cocoon is thick and gray or black, and the summer cocoons are often thin and white with a light band around the middle.

The adults will feed on honey or sweetened water, but not on the body or blood of the host larvae. They may live as long as 111 days at a low temperature. The newly-emerged female will have about 100 fully formed eggs in the ovaries and ducts, and can deposit altogether as many as 300 eggs.

The egg is deposited in the body cavity of the young host larva, and the parasite larva lives free in the host, destroying it by absorbing the internal organs as the larva approaches maturity. The parasite emerges from the old borer skin and spins its cocoon in the host tunnel. Development proceeds slowly during hibernation.

Generally there is but one generation a year; the adults from the overwintering cocoons emerge from March to June and lay their eggs in the earliest appearing corn-borer larvae in July or August. Only the latest emerging adults live long enough to find *Pyrausta* larvae in which to oviposit, for by the time these appear the majority of *Eulimneria* adults have probably died. So far as the writers know there is no intermediate host. The larval growth takes place in summer, and the overwintering cocoons are spun in September or October.

Eulimneria crassifemur clings feebly to a seasonal rhythm, but this can be broken by a considerable fluctuation in the temperature factor. Thus temperature seems to be the most important single climatic factor influencing its range. The parasite does not occur on the French Riviera, as the prevailing high temperature probably prevents a successful seasonal rhythm. The fact that it occurs around Naples may be accounted for on the theory that it migrates annually, or at least at intervals, from the higher mountain valleys.

Eulimneria crassifemur is found in the corn borers in both corn and *Artemisia*. The maximum parasitism of the host that has been recorded in Europe is 27 per cent.

Limiting factors that may hinder the abundance of this species are a lack of perfect synchronization between host generations and parasite generations, superparasitism, or the laying of more than one egg in the same host, and cold, wet weather during the oviposition period. A few secondary parasites have been reared, but they are negligible as far as influencing the numbers of the parasite is concerned.

LITERATURE CITED

- (1) ASHMEAD, W. H.
1900. SOME CHANGES IN GENERIC NAMES IN THE HYMENOPTERA. *Canad. Ent.* 32: 368.
- (2) CAFFREY, D. J., and WORTHLEY, L. H.
1927. A PROGRESS REPORT ON THE INVESTIGATIONS OF THE EUROPEAN CORN BORER. U. S. Dept. Agr. Bul. 1476, 155 p., illus.
- (3) CUSHMAN, R. A.
1926. SOME TYPES OF PARASITISM AMONG THE ICHNEUMONIDAE. *Ent. Soc. Wash. Proc.* 28 (2): 25-51, illus.
- (4) DALLA TORRE, C. G. DE.
1901-02. CATALOGUS HYMENOPTERORUM HUCUSQUE DESCRIPTORUM SYSTEMATICUS ET SYNONYMICUS. v. 3, 1141 p. Lipsiae.
- (5) PAILLOT, A.
1924. LA LYDA DU PÊCHER. ÉTUDE BIOLOGIQUE-MÉTHODES DE DESTRUCTION. *Ann. Épiphyties* 10: [147]-237, illus.
- (6) ROUBAUD, E.
1922. ÉTUDES SUR LE SOMMEIL D'HIVER PRÉ-IMAGINAL DES MUSCIDES. LES CYCLES D'ASTHÉNIE ET L'ATHERMOBIOSE RÉACTIVANTE SPÉCIFIQUE. *Bul. Biol. France et Belg.* 56: [455]-544, illus.

- (7) SCHMIEDEKNECHT, O.
1907. DIE HYMENOPTEREN MITTELEUROPAS . . . 804 p., illus. Jena.
- (8) SCHWANGART, F.
1918. ÜBER REBENSCHÄDLINGE UND-NÜTZLINGE. V. DIE SCHLUPFWESPEN DER TRAUBENWICKLER ZUCHTERGEBNISSE. Centbl. Bakt. [etc.] 48: 543-558.
- (9) SEURAT, L. G.
1899. CONTRIBUTIONS À L'ÉTUDE DES HYMÉNOPTÈRES ENTOMOPHAGES. 159 p., illus. Paris. (Thèse, Faculté des Sciences.)
- (10) SMITS VAN BURGST, C. A. L.
1919. SLUIPWESPEN, GEKWEKTE UIT DE DENNENLOTTRUPS (EVETRIA BUOLIANA SCHIEFF.); PERILAMPUS BATAVUS N. SP. Tijdschr. Ent. 61: [143]-146.
- (11) SPENCER, H.
1926. BIOLOGY OF THE PARASITES AND HYPERPARASITES OF APHIDS. Ann. Ent. Soc. Amer. 19: 119-153, illus.
- (12) SZÉPLIGETI, G. V.
1911. HYMENOPTERA, FAM. IGHNEUMONIDÆ GRUPPE MESOCHOROIDÆ (OPHIONOIDÆ PART) SUBFAM. LIMNERINÆ, MESOCHORINÆ, ADELOGNATHINÆ, PLECTISCINÆ, BANCHINÆ, NEOMESOCHORINÆ, MEGACERINÆ und PANISCINÆ. In Wytsman, P. Genera Insectorum, Fasc. 114, 100 p., illus. Brussels.
- (13) THOMSON, C. G.
1887. OPUSCULA ENTOMOLOGICA. Fasc. 11, p. 1041-1182. Londæ.
- (14) THOMPSON, W. R.
1922. THEORIE DE L'ACTION DES PARASITES ENTOMOPHAGES. 'LES FORMULES MATHÉMATIQUES DU PARASITISME CYCLIQUE. Compt. Rend. Acad. Sci. [Paris] 174: 1201-1204.
- (15) ———
1927. A METHOD FOR THE APPROXIMATE CALCULATION OF THE PROGRESS OF INTRODUCED PARASITES OF INSECT PESTS. Bul. Ent. Research 17: 273-277.
- (16) ———
1927. ON THE EFFECT OF METHODS OF MECHANICAL CONTROL ON THE PROGRESS OF INTRODUCED PARASITES OF INSECT PESTS. Bul. Ent. Research 18: 13-16.
- (17) TIMBERLAKE, P. H.
1912. TECHNICAL RESULTS FROM THE GIPSY MOTH PARASITE LABORATORY. V. EXPERIMENTAL PARASITISM: A STUDY OF THE BIOLOGY OF LIMNERIUM VALIDUM (CRESSON). U. S. Dept. Agr., Bur. Ent. Tech. Ser. 19 (pt. 5): 71-92, illus.
- (18) TOTHILL, J. D.
1922. THE NATURAL CONTROL OF THE FALL WEBWORM (HYPHANTRIA CUNEA DRURY) IN CANADA TOGETHER WITH AN ACCOUNT OF ITS SEVERAL PARASITES. Canada Dept. Agr. Bul. (n. s.) 3 (Ent. Bul. 19), 107 p., illus.
- (19) TRÄGÅRDH, I.
1923. SKOGSENTOMOLOGISKA BIDRAG II. Meddel. Statens Skogsförsöksanst. [Sweden] 20: [401]-424, illus.



THE POSSIBLE TOXICITY OF GRAIN-SORGHUM SMUTS¹

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INTRODUCTION

The grain sorghums have proven to be especially adapted to some of the more arid sections of this country where they are grown and fed in large quantities to livestock. Unfortunately many members of the group are subject to kernel smut infections. These infections can be partially avoided by proper treatment of the seed with copper carbonate or other fungicides, but the treatment is often omitted, with the result that in some years as high as 70 per cent of the heads in certain fields are affected. In the same sections of the country the small grains are often attacked by the fungus ergot, which is distinctly toxic to livestock.

When the dried heads are handled or threshed, the smut spores are liberated in the form of a very fine dust. The writers have noted peculiar physiological reactions upon its inhalation. The upper pulmonary tract was sensitive to the passage of air, the heart action seemed to be increased, these symptoms being followed a few hours afterwards by headache and a partially nauseated feeling. For these and other reasons, it has been thought that deleterious results might be associated with the consumption of smut spores. Livestock feeders have often hesitated to use feed thus affected, and when death did occur among the cattle, it has been attributed to this cause.

An investigation of the literature has failed to reveal any definite information in regard to the effect of grain-sorghum smut on livestock. The only related work consists of studies of corn smuts. Pammel³ states that corn smut is supposed to be poisonous to cattle, in some forms ergotin is found, while the Bureau of Animal Industry⁴ finds that smut is not toxic to heifers. This finding is in accord with that of Smith⁵, who fed smutty corn to cows without injury, in fact the cows seemed to relish it. Henry⁶ fed large quantities to cows for a considerable time. They fattened and did well until one unaccountably died. Hutyra and Marek⁷ state that smut produces gout in chickens. However, the smut of corn is of an entirely different variety, and the sorghum smut might have properties in common with the toxic smuts of small grains.

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² The writers wish to express their appreciation to H. H. Fennell, in charge of the experiment station at Goodwell, for his assistance in gathering and classifying the smut.

³ PAMMEL, L. H. A MANUAL OF POISONOUS PLANTS, CHIEFLY OF EASTERN NORTH AMERICA, WITH BRIEF NOTES ON ECONOMIC AND MEDICINAL PLANTS, AND NUMEROUS ILLUSTRATIONS. 214 p., illus. Cedar Rapids, Iowa. 1911.

⁴ MOORE, V. A., and SCHWEINITZ, E. A. DE. CORNSTALK DISEASE AND RABIES IN CATTLE . . . U. S. Dept. Agr., Bur. Anim. Indus. Bul. 10, 92 p., illus. 1896.

⁵ SMITH, C. D. FEEDING CORN SMUT TO DAIRY COWS. Mich. Agr. Expt. Sta. Bul. 137, p. [41]-46. 1896.

⁶ HENRY, W. A. FEEDING CORN SMUT. Wis. Univ. Board of Regents Ann. Rpt. 1881: 50-54. 1881. (Pub. Doc. 6.)

⁷ HUTYRA, F., and MAREK, J. SPEZIELLE PATHOLOGIE UND THERAPIE DER HAUSTIERE. Ed. 6, v. 3, p. 187. Jena. 1922.

For these reasons it has seemed advisable to conduct a series of experiments in order to obtain some definite information in regard to the effect of grain-sorghum smut on the health of animals when it is present in their feed.

EXPERIMENTAL METHODS AND PROCEDURE

During the year 1927, some sorghum fields were visited in which as high as 70 per cent of the heads were smut-infected to a greater or less degree. Before harvesting the crop, heads in which practically all the kernels were filled with spores were gathered and dried. These heads were threshed by hand and the smut spores separated by the use of fine screens. This smut weighed 34.1 per cent as much as the grain in an equal number of noninfected heads. Partial analysis of the air-dried material gave the following results:

	Per cent
Water.....	9.00
Ash.....	8.42
Proteins.....	13.5
Dextrose.....	1.03
Invert sugar.....	.07
Lost with ether extract.....	.76
Lost with 95 per cent alcohol following ether extract.....	.66
Water soluble following ether and alcohol extract.....	20.26

FEEDING EXPERIMENTS WITH RATS

In order to determine whether or not the smut spores would be injurious when fed to animals, experiments were planned whereby a comparison could be made of the growth and reproduction of various kinds of animals receiving a complete well-balanced basal ration and a second series receiving the same rations supplemented with various amounts of the smut spores. In the first tests rats were used as experimental animals. Healthy young rats about 4 weeks of age were chosen and placed in the cages regularly used for nutrition tests, care being exercised to place a lot comparable in litter origin, sex, and size in each cage. A ration was planned so as to be similar to the one consumed by animals eating the whole grain and yet be complete in proteins, vitamins, and minerals.

The percentage constitution of the basal ration was as follows: Kafir, 80; tankage, 8; ground alfalfa, 5; NaCl, 1; CaCO₃, 1; and cod-liver oil, 5.

One lot of rats used as a control series were fed this ration throughout the experiment and records were made of their growth, age of reproduction, the number of young, and the development of offspring. Similar lots received the same ration in which varying quantities of smut spores replaced like quantities of carbohydrates in such manner as would cover the usual range of contaminated heads.

The general results are to be found in the growth charts. Figure 1 represents the growth of animals receiving the basal ration. Growth and reproduction were normal in every case.

Figure 2 represents the growth and reproduction of animals receiving the basal ration supplemented with 8 per cent smut. It will be observed that the growth was fully as satisfactory as in the case of those receiving the basal ration alone. It will be further observed that the females became pregnant and gave birth to young. The

ergot of small grains seems to cause an abnormality in pregnant mothers, abortion being common. The number of young rats in the litter was as great as is usually found in normal animals. There were some losses by death among the lots receiving the basal ration and slightly higher death rates prevailed among those receiving the smut. An examination, however, failed to reveal any symptoms of toxic conditions. It was concluded that the death of some young might be due to the fact that the fiber content of the feed was higher than is desirable for rat feeding.

Figure 3 records similar results for smut feeding in the second generation. Records of third and fourth generation animals, though not charted in the

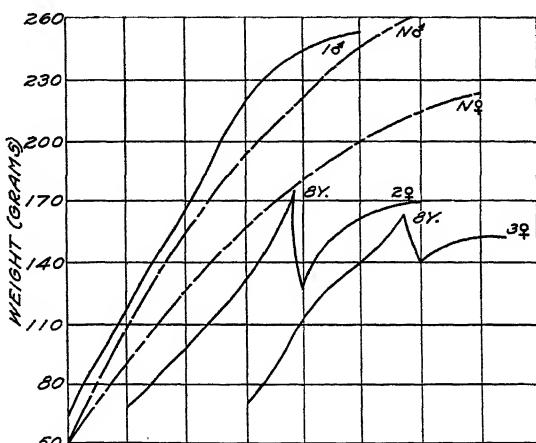


FIGURE 1.—Growth and reproduction of rats on a complete basal ration. The solid lines represent the growth of the experimental animals, the broken lines that of normal animals. N and Y signify normal and young, respectively. Spaces between ordinates represent 4-week periods

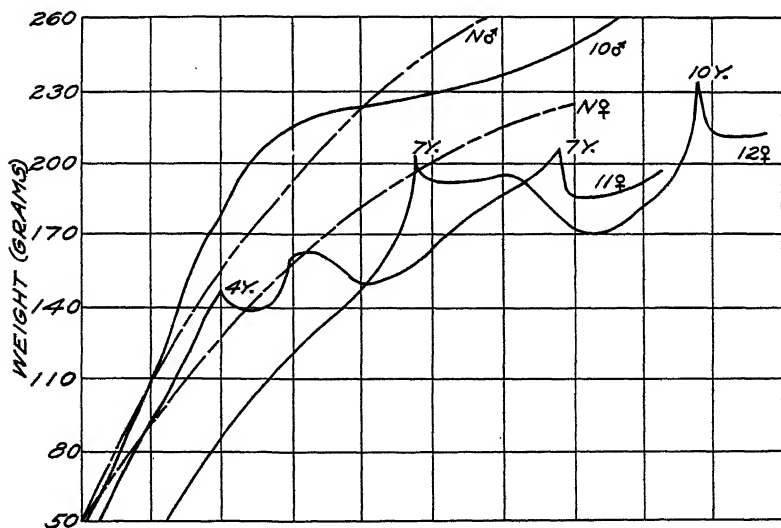


FIGURE 2.—Growth and reproduction of rats on a complete basal ration to which 8 per cent of sorghum-smut spores had been added, replacing carbohydrates in like amount. The solid lines represent the growth of the experimental animals, the broken lines that of normal animals. N=normal, Y=young. Spaces between ordinates represent 2-week periods

figures, have been likewise made, and there was no evidence of deleterious effects from feeding smut spores.

EXPERIMENTS WITH OTHER SMALL ANIMALS

It was then decided that even though the smutty grain might not be injurious to rats it might be toxic to other animals, so experiments were planned using both rabbits and guinea pigs. In this case the percentage composition of the basic ration was as follows: Ground kafir, 59; wheat, 30; alfalfa, 5; milk powder, 5; NaCl, 1; and CaCO_3 , 1.

Green lettuce or cabbage was added twice a week. Six per cent smut was substituted for an equal amount of kafir in the smut test rations. Both the guinea pigs and the rabbits ate the rations readily. No apparent difference could be observed between the animals consuming the basal ration and the smutty food. Growth and reproduction were normal. The average increase in weight per week was 18 gm. for guinea pigs and 134 gm. for the rabbits, this increase being

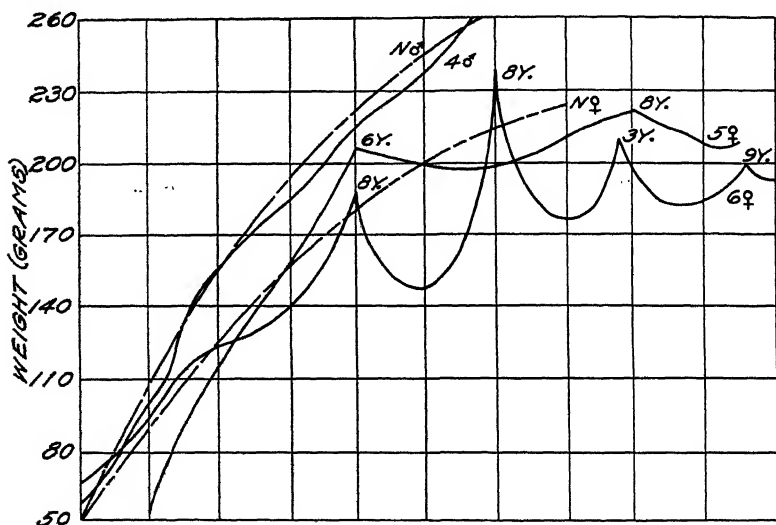


FIGURE 3.—Growth and reproduction of second-generation rats which received the complete basal ration to which 10 per cent of sorghum-smut spores had been added, replacing carbohydrates in like amount. The solid lines represent the growth of the experimental animals, the broken lines that of normal animals. N=normal, Y=young. Spaces between ordinates represent 2-week periods.

practically uniform throughout the test period. One of the rabbits gave birth to nine young.

Inasmuch as kafir furnishes an ideal feed for chickens, an extended study is being made with laying hens and growing chickens. Even though the smut was present in such large quantities that the chickens' throats became black with it, no deleterious effects have so far been noted.

FEEDING EXPERIMENTS WITH HORSES AND COWS

Having demonstrated under carefully controlled conditions that the smut spores seemed to have no ill effect on small experimental animals, it was next desirable to observe the effects on farm animals as fed under farm conditions, or when the rations were somewhat inadequate, as is often the case under these conditions. A farmer was found who had broadcast his sorghum seed. The sorghum plants were somewhat thick, the heads were small, and fully 70 per cent of

them were smut-infected. The entire plant was cut and bound, the seed and smut remaining in the head, and fed to horses, milk cows, and young cattle.

The horses were mature animals used for farm work and received only the sorghum fodder including the smutty heads, together with yellow ear corn, salt, and water. After 12 weeks of such a limited diet, no ill effect could be observed. During a greater portion of the time the horses were used for heavy hauling.

The milk cows were fed a pound of soybean meal a day, as much of the sorghum fodder as they desired, together with salt and water. Nine young cattle received only fodder, containing the smutty grain in head, salt and water. Six of these gave birth to normal calves at the normal time. All of the animals maintained themselves as well as could be expected on so limited a ration. It is thought that the tests demonstrate beyond doubt that the smut possesses no toxic principles when fed in as concentrated a form as ordinarily found in the field, and especially was this the case since the animals were fed a ration the efficiency of which was limited by the character and quantity of its proteins and vitamins. It is held to be quite generally true that when animals are receiving a somewhat inadequate ration they become more susceptible both to diseases and toxic substances than when better nourished.

SUMMARY AND CONCLUSIONS

Certain types of grain sorghums often have as high as 70 per cent of the heads affected with kernel smuts which replace the grain kernels.

Biological tests using rats, guinea pigs, and rabbits failed to reveal any deleterious results in growth, reproduction, and rearing of young when as high as 10 per cent of smut spores replaced an equal amount of carbohydrates in an otherwise adequate diet.

Chickens were fed satisfactorily on rations prepared from smutty seed.

Horses, cows, and young cattle were fed smutty sorghum grain and fodder without displaying any symptoms of toxicity.

During the observation of all these animals (over 65 in number) no sickness or deaths occurred. Young animals grew as well as the controls. Old animals maintained their weight. Reproduction took place at normal periods with four types of animals and neither the egg production of hens nor the milk production of cows was altered by feeding sorghum smut in a form as concentrated as it occurs in the field. This was true with larger farm animals even when their ration was somewhat inadequate.

INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN WHITE ODESSA WHEAT¹

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INTRODUCTION

The inheritance of resistance to bunt, *Tilletia tritici* (Bjerk.) Wint., in Martin and Hussar wheats has been described in previous publications (2, 3).² Since the literature pertinent to the inheritance of resistance to bunt has been reviewed and discussed in these publications, it will not be repeated here.

Martin and Hussar were the only two varieties of wheat found to be completely resistant to bunt in an extensive varietal test conducted in the Pacific Coast States (10). However, a few other varieties were highly resistant, usually producing less than 10 per cent of diseased heads. White Odessa (C. I.³ 4655) was such a variety, with an average of only 2 per cent of bunted heads at the Washington, Oregon, and California Agricultural Experiment Stations, and an average of 2.3 per cent of bunted heads at the California station. However, the selection of White Odessa used in this investigation has been completely resistant to bunt at Davis, Calif., in the 3-year period beginning in 1926.

In 1925 White Odessa was crossed with White Federation, which is very susceptible to bunt. The data obtained in a study of the F₂ and F₃ progenies are described in this paper.

The inoculum was from the same collection of bunt as that used in 1920 and 1921. The original collection may have consisted of two or more physiologic forms, one of which could attack White Odessa slightly. It is conceivable that this form may have been lost in propagating the inoculum on White Federation wheat. In any case, the genetic difference between a completely resistant selection of White Odessa and one which produces 2 per cent of bunted heads probably is not very great.

White Federation may be considered completely susceptible as compared with White Odessa. Three rows contained an average of 80.8 per cent of bunted plants in 1926, 13 rows an average of 66.6 per cent in 1927, and 12 rows an average of 68.9 per cent in 1928. The average for the 9-year period beginning in 1920 was 71.4 per cent of bunted plants.

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. Conditions there were especially suitable for these investigations because relatively high bunt infection can be obtained when wheat is sown in the fall. Both

¹ Received for publication July 27, 1929; issued February, 1930. Results of cooperative investigations between the Bureau of Plant Industry, U. S. Department of Agriculture, and the California Agricultural Experiment Station.

² Reference is made by number (italic) to "Literature cited," p. 359.

³ C. I. indicates a serial number of the Office of Cereal Crops and Diseases.

spring and winter varieties may be seeded at that time of year without any danger of winterkilling and with the assurance that both types will mature in the following summer.

In the present investigation the seeds were thoroughly blackened with bunt by placing an excess quantity of the spores with the wheat in a glass container and shaking vigorously. The inoculum was originally collected by W. W. Mackie in 1917 on Little Club wheat in the Montezuma Hills district of Solano County, Calif.

PARENTS AND METHODS

White Odessa wheat (C. I. 4655) produced 2 per cent of bunted heads at Davis in 1920 and 2.7 per cent in 1921. From 1921 to 1925 this variety was propagated each year from seeds from single plants in the parent wheat nursery and was not subjected to bunt infection. In 1925 the flowers of one head were crossed with pollen from White Federation wheat. The other heads on that plant have been the source of seed of White Odessa wheat used since that time.

During the 3-year period beginning in 1926 this strain of White Odessa has been completely free from bunt, as compared with an average of 2.3 per cent of diseased heads produced in 1920 and 1921 under similar conditions. It is entirely possible that the original lot of seed of this variety was made up of two or more biotypes and that the plant used for this cross happened to be a completely resistant one.

Mackie supplied the writer with a quantity of inoculum which he had propagated on Little Club wheat in the botany garden at Berkeley, Calif. Since 1919 the writer has grown this same collection on White Federation wheat at Davis. The inoculum, therefore, has been propagated from one original collection of bunt. This was done not primarily because it was suspected that physiologic forms of bunt existed, but because this offered an easy and definite source of spores. More recently, Faris (4), Rodenhiser and Stakman (9), Gaines (6), and Rodenhiser (8) have reported physiologic forms of this fungus. The fact that the same collection of bunt has been used continuously at Davis makes it quite certain that the same form or mixture of forms was employed. This is indicated also by the fairly constant way in which the parent material has reacted to inoculation with this collection.

The seeds were spaced from 2 to 3 inches apart in rod rows 1 foot apart. The entire nursery was sown during a period of three or four days, in order to avoid the effects of changing conditions of temperature and moisture. The nursery always was sown in a field in which no wheat had been grown during the previous year and which consequently was almost entirely free from volunteer plants.

At harvest time the plants in each row were pulled and separated into two piles, one bunt free and the other bunted. The total number of plants and the number of bunted plants were recorded and the percentage of bunted plants was calculated from these. A plant was classified as bunted if it showed even a trace of the disease.

EXPERIMENTAL RESULTS

The cross White Odessa \times White Federation was made in 1925 and the F_1 generation was grown the following year. F_1 seeds were not inoculated because of the small number available.

Some of the F_2 seeds were treated with copper carbonate to protect them from bunt infection and thus insure a supply of seeds for the F_3 generation. The other F_2 seeds were inoculated in order to get some indication as to the number of factors present and to find out how many bunted plants to expect in F_3 rows of the same genotype.

F_2 data do not permit a Mendelian analysis, because some susceptible plants escape infection. Even in the most susceptible varieties rarely ever are 100 per cent of the plants diseased. Apparently the bunt-free plants in such a row merely have escaped infection, because they do not differ in resistance from the unselected variety. Data collected in F_2 are recorded in Table 1.

TABLE 1.—Percentage of bunted plants in the parents and in the F_2 generation of the cross *White Odessa*×*White Federation*, when grown in the field at University Farm, Davis, Calif., in 1927

Parent or cross	Number of plants		Percentage of bunted plants
	Total	Bunted	
White Odessa.....	258	0	0
White Federation.....	579	385	66.5
White Odessa×White Federation.....	422	94	22.3

There were 22.3 per cent of bunted plants in the F_2 of *White Odessa*×*White Federation*, which is very near the 25 per cent expected on the basis of a single dominant factor for resistance. *Martin*×*White Federation* produced 17.2 per cent of bunted plants in F_2 (2). It was shown that enough susceptible plants had escaped infection to bring this figure into satisfactory agreement with the 25 per cent expected. In the case of *White Odessa*×*White Federation* either fewer susceptible plants escaped infection or more resistant and heterozygous plants became infected. That resistant and heterozygous plants occasionally may become infected will be shown later in this paper. F_2 data, then, indicate that *White Odessa* has a single dominant factor for resistance to bunt, and that heterozygous F_3 rows should contain an average of about 22 per cent of bunt.

In F_3 299 rod rows were grown from 299 F_2 plants which had been protected from bunt infection by seed treatment with copper carbonate. As already pointed out, the classification of F_2 plants on the basis of F_3 rows is more reliable than classification in F_2 . The F_3 data are recorded in Table 2.

TABLE 2.—Distribution of the parent and the F_3 rows of the cross *White Odessa*×*White Federation* into 5 per cent classes for bunt infection, when grown at Davis, Calif., in 1928

Parent or cross	Distribution of rows by percentage classes of bunt infection																		Total number of rows		
	0	<5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	70-75	75-80	80-85		85-90	90-95
White Odessa	12																				12
White Federation											1			2	1	3		3	2		12
White Odessa × White Federation	57	13	11	21	33	47	29	12	5	2	8	0	4	3	6	12	15	16	4	1	299

The rows having from 0 to just less than 5 per cent of bunted plants were separated into those with none and those with some but less than 5 per cent of bunted plants, because of the special interest in the former. The nature of the distribution may be seen more readily in Figure 1. The numbers of rows under the three modes agree satisfactorily with the 1:2:1 ratio. There are 76 rows set off by the first minimum where 74.75 are expected. Between the first and second minima there are 162 rows where 149.5 are expected. This is a deviation of 12.5 ± 5.83 , which is 2.1 times the probable error. From the second minimum on there are 61 rows where 74.75 are expected, a deviation of 13.75 ± 5.05 , which is 2.7 times the probable error. The agreement is satisfactory, although not close. If the 8 rows with 45 to 50 per cent of bunt are considered as susceptible, the deviations all are within about one time the probable error. The

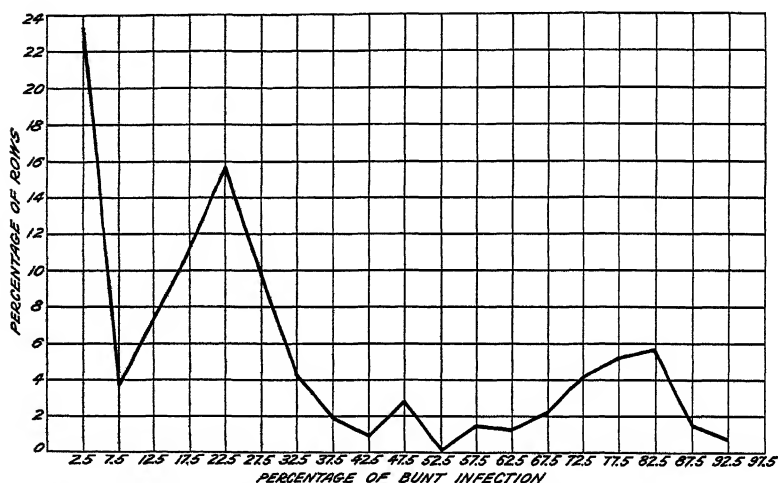


FIGURE 1.—Distribution of F_2 plants on the basis of F_3 rows of the cross White Odessa \times White Federation into 5 per cent classes for bunt infection

minima should not be thought of as marking with absolute accuracy the divisions between phenotypes. However, it is interesting to note that in the curves for Martin \times White Federation, Martin \times Hard Federation, and White Odessa \times White Federation the first minimum for each curve has fallen at 7.5 and the second one very near 50 per cent.

The 76 resistant rows were made up of 57 that were completely resistant, and 19 that contained a low percentage of bunt. Other data show that modifying factors may be responsible for the presence of a slight percentage of bunted plants in rows that were expected to be completely resistant like the resistant parent. In some cases the one or two diseased plants in a row may have been due to mechanical mixtures that occurred at the time of threshing or seeding.

The 162 F_3 rows from heterozygous F_2 plants averaged 23.5 per cent of bunted plants, as compared with 22.3 per cent in 10 F_2 rows. The F_3 rows contained from 7.5 to 47.5 per cent of bunted plants, as compared with 15 to 35 per cent in F_2 rows. Had as large a number of F_2 rows been grown, it is possible that similar extremes of bunt

infection would have been obtained. The seeds of bunt-free plants and partly bunted plants of F_2 row No. 355, which produced 15.8 per cent of bunted plants, and F_2 row No. 357, which produced 35.1 per cent, were saved and the F_3 was grown to see if these two extreme rows would come into line when the F_2 plants were classified according to bunted plants produced in F_3 rows. That there is satisfactory agreement with the single-factor hypothesis may be seen from the data in Table 3.

TABLE 3.—Distribution of F_3 rows of the cross *White Odessa* × *White Federation* from F_2 rows Nos. 355 and 357 into 5 per cent classes for infected plants, when grown at Davis, Calif., in 1928

5 per cent infection classes	Row No. 355					Row No. 357						
	Number of plants				Dif- ference Prob- able error	Number of plants				Dif- ference Prob- able error		
	Observed by—		Ex- pected	Difference		Observed by—		Ex- pected	Difference			
	Classes	Modes				Classes	Modes					
0-----	5	}	6	9.5	-3.5±1.80	1.94	15	}	17	14.25	+2.75±2.20	1.25
<5-----	1						2					
5-10-----	2	}	20	19	+1±2.08	0.48	5	}	26	28.50	-2.50±2.55	0.98
10-15-----	5						7					
15-20-----	5						6					
20-25-----	7						4					
25-30-----	5						2					
30-35-----	1						2					
35-40-----												
40-45-----												
45-50-----												
50-55-----												
55-60-----												
60-65-----	1	}	12	9.5	+2.5±1.80	1.39	1	}	14	14.25	-0.25±2.20	0.11
65-70-----	1						1					
70-75-----	2						1					
75-80-----	2						1					
80-85-----	2						1					
85-90-----	1						1					
90-95-----	1											
Total--	38						57					

* Includes two plants that were completely diseased in F_2 and that therefore produced no good seed.

† Includes nine plants that were completely diseased in F_2 and that therefore produced no good seed.

If the plants that were completely smutted in F_2 are classed as susceptible and are added to the F_3 rows that are classed as susceptible, the agreement with the 1:2:1 ratio in both families is satisfactory. The greatest deviation is only 1.94 times the probable error.

In family No. 355 there were 2 completely bunted and 4 partly bunted plants in a row of 38 plants. Of the 4 partly bunted plants 3 proved to be homozygous susceptible and the other heterozygous. In addition, there were 7 susceptible plants which had escaped infection in F_2 .

Family No. 357 produced 9 completely bunted and 11 partly bunted plants. Of the 11 partly bunted plants 3 were found to be susceptible, 6 heterozygous, and 2 resistant. There was one bunted head in F_2 on each of the 2 plants that proved to be resistant. Two susceptible plants had escaped infection.

There are several reasons, then, for the great differences in the percentage of bunted plants produced by heterozygous rows. In some

cases a large proportion of susceptible plants escape infection while in other rows only a few escape. In other cases apparently a rather large proportion of heterozygous plants become infected. Finally, in addition to mechanical mixture, there are resistant plants that become infected. The latter case is due fairly certainly to modifying factors. To what extent other differences are due to modifying factors is not known. Environmental influences may be active.

The group of susceptible rows, as indicated in Figure 1, produced an average of 75.6 ± 0.66 per cent of bunted plants as compared with 68.9 ± 2.15 per cent for the White Federation parent. The difference is 6.7 ± 2.25 , which is 2.98 times the probable error. If the eight rows with 45 to 50 per cent bunt are considered as susceptible, the average for the susceptible group is 72.4, giving a difference of 3.5.

The segregation of F_2 plants, determined on the basis of the percentage of bunted plants in F_3 rows, shows that White Odessa differs from White Federation in one main dominant factor for resistance to bunt. The distribution of rows, as shown in Figure 1, is quite similar to that obtained for Martin \times White Federation (2, fig. 2). Whether or not the factor for resistance in White Odessa is the same as the one in Martin is not known at present, but this point is being investigated.

DISCUSSION AND SUMMARY

In a previous publication (2) the writer has shown that Martin wheat differs from such susceptible varieties as White Federation and Hard Federation in one main dominant factor for resistance. He has also shown that Hussar (2, 3) differs from such susceptible varieties in two main factors for resistance. One of these is the same factor as that found in Martin; the other allows bunt to develop on about half of the heterozygous plants. In the present paper data are presented which indicate that White Odessa differs from White Federation in one main factor for resistance, and that this factor is similar in its effect to the one found in Martin. Whether or not the factor in White Odessa is identical with that in Martin is not at present known.

Gaines (5) believes that resistance to bunt in such varieties as Martin, Hussar, and White Odessa probably is due to a large number of unit factors, the cumulative effect of which is to make the total result appear dominant, and that a lesser number would give a recessive effect.

McRostie (7), Aamodt (1), and others have shown the necessity of using known physiologic forms of the causal fungi in genetic studies of resistance to plant diseases. The same collection of bunt has been propagated and used continuously at Davis, but whether this is made up of more than one physiologic form is not known. A satisfactory set of differential hosts is not yet available for determining this point.

There may be other factors for resistance to bunt in Martin, Hussar, and White Odessa which would become apparent in the presence of other physiologic forms of this organism.

An attempt is being made to isolate as many different factors for resistance as possible. Once the different factors are isolated, their reaction to the different physiologic forms of bunt may be determined.

Some progress has been made in breeding bunt-resistant wheats. No doubt the effective breeding of bunt-resistant wheats in the

presence of physiologic strains of this disease will depend in some cases on bringing together two or more factors for resistance.

LITERATURE CITED

- (1) AAMODT, O. S.
1923. THE INHERITANCE OF GROWTH HABIT AND RESISTANCE TO STEM RUST IN A CROSS BETWEEN TWO VARIETIES OF COMMON WHEAT. *Jour. Agr. Research* 24: 457-470, illus.
- (2) BRIGGS, F. N.
1926. INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI* (BJERK.) WINTER, IN WHEAT. *Jour. Agr. Research* 32: 973-990, illus.
- (3) ———
1929. THE INHERITANCE OF THE SECOND FACTOR FOR RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN HUSSAR WHEAT. *Jour. Agr. Research* 40: 225-232, illus.
- (4) FARIS, J. A.
1924. FACTORS INFLUENCING THE INFECTION OF WHEAT BY *TILLETIA TRITICI* AND *TILLETIA LEVIS*. *Mycologia* 16: 259-282.
- (5) GAINES, E. F.
1925. THE INHERITANCE OF DISEASE RESISTANCE IN WHEAT AND OATS. *Phytopathology* 15: [341]-349.
- (6) ———
1928. NEW PHYSIOLOGIC FORMS OF *TILLETIA LEVIS* AND *T. TRITICI*. *Phytopathology* 18: 579-588.
- (7) McROSTIE, G. P.
1919. INHERITANCE OF ANTHRACNOSE RESISTANCE AS INDICATED BY A CROSS BETWEEN A RESISTANT AND A SUSCEPTIBLE BEAN. *Phytopathology* 9: [141]-148.
- (8) RODENHISER, H. A.
1928. PHYSIOLOGIC SPECIALIZATION IN SOME CEREAL SMUTS. *Phytopathology* 18: 955-1003, illus.
- (9) ——— and STAKMAN, E. C.
1927. PHYSIOLOGIC SPECIALIZATION IN *TILLETIA LEVIS* AND *TILLETIA TRITICI*. *Phytopathology* 17: 247-253, illus.
- (10) TISDALE, W. H., MARTIN, J. H., BRIGGS, F. N., MACKIE, W. W., WOOLMAN, H. M., STEPHENS, D. E., GAINES, E. F., and STEVENSON, F. J.
1925. RELATIVE RESISTANCE OF WHEATS TO BUNT IN THE PACIFIC COAST STATES. U. S. Dept. Agr. Bul. 1299, 30 p.



ISOLATION AND PURIFICATION OF THE ALCOHOL-SOLUBLE PROTEIN (PROLAMIN) OCCURRING IN ENGLISH RYEGRASS (*LOLIUM PERENNE*)¹

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INTRODUCTION

In spite of considerable difficulties encountered in dealing with plant proteins, their great significance as carriers of the most important vital functions in plant, animal, and man, together with their high nutritive value, makes it well worth the time and labor to throw additional light on their occurrence as well as on their physical and chemical properties. So far as English ryegrass is concerned, a careful perusal of the literature reveals the fact that knowledge concerning its chemical constituents is extremely meager. At present all that is known is the analysis by Deetz (6)² of the fat, ash, and nitrogen content of ryegrass plants in various stages of their development, and the reports of Gautier (7) and Arnaud (1) concerning their chlorophyll and carotin content, respectively. When the work of Schulze and his collaborators (30) on some nitrogenous compounds of the ryegrass is also mentioned, all of the literature dealing with its chemical constituents appears to be exhausted so far as the writer is aware.

We are indebted to Osborne for most of our knowledge concerning the alcohol-soluble proteins in plants. From his numerous researches, of which a few only can be mentioned here, and partly from those of Ritthausen (29), it is known that gliadin is present in the seeds of wheat (*Triticum vulgare*) (25, 26, 27) and rye (*Secale cereale*) (21, 26), hordein in barley (*Hordeum vulgare*) (22, 27), zein in maize (*Zea mays*) (23), and that a prolamins not named by Osborne is present in the seed of oats (*Avena sativa*) (20). Johns and Brewster as well as Hoffman have also reported the occurrence of prolamins in the seeds of kafir (*Andropogon sorghum*) (19) and of rice³ (*Oryza sativa*) (10), respectively. Somewhat later the discovery of a prolamins in English ryegrass was reported by Jodidi and Peklo (17). The work on the aleurone layer by Peklo (28) is of interest in this connection. Yet in the joint paper the proof of the occurrence of prolamins in the ryegrass was based exclusively on the estimation of the nitrogen that could be extracted from the ryegrass with dilute alcohol. The prolamins itself was not isolated and hence could not be purified, analyzed, characterized by its reactions, or otherwise identified. It is with these problems that the present paper is concerned.

METHODS AND RESULTS

The protein nitrogen was determined according to Stutzer's method (31) as applied by the writer (12, 13, 14, 15, 16, 18) in previous investi-

¹ Received for publication Aug. 9, 1929; issued February, 1930.

² Italic numbers in parentheses refer to "Literature cited," p. 369.

³ Only a trace of a protein soluble in hot alcohol was found in the rice kernel.

gations. Sulphur was estimated by oxidizing the substance with a mixture of potassium nitrate and potassium hydroxide and precipitating the resulting sulphuric acid as barium sulphate.

FIRST ISOLATION AND PURIFICATION OF THE PROLAMIN

Five hundred grams of English ryegrass flour was extracted with 2 liters of 70 per cent alcohol at 55° C. for 24 hours, a deep brownish-red extract resulting. The extraction mixture was then strained through a cloth at about 50° and the residue squeezed out dry in a screw press. The resulting meal was extracted again with 1½ liters of 70 per cent alcohol at 55° for another 24 hours, strained through cloth, and the residue squeezed out again. The second extract was of a light-yellow color. The two extracts were united, filtered clear, and concentrated in the water bath at low temperature. On the surface of the liquid thin, transparent, brittle, brownish-yellow films formed, which were readily soluble in dilute alcohol, while there settled on the bottom of the dish a jellylike viscid mass which could be drawn to long threads or sheets. The substance thus concentrated was allowed to cool overnight. The following morning an attempt to filter the cooled residue through a filter on a Büchner funnel with suction failed completely. Filtration was extremely slow even through cheesecloth on a Büchner funnel. For this reason the mother liquor, which had a brown color due in part to anthocyanin as shown by its reactions, was decanted as much as possible and the residue washed with some water, after which it was centrifuged. The cake-like substance which remained in the centrifuge tubes was apparently quite impure.

The cakes were next treated in a flask with anhydrous ether for a period of 4 hours, the flask being shaken frequently. The ether, which assumed a yellow color due to the extracted fat, was decanted and discarded. The resulting cakes were then minced and treated with about 500 c. c. of absolute alcohol for 24 hours, the flask being shaken from time to time. The alcohol assumed a pretty wine-red color due to the coloring matter and the fat taken up, while the substance itself became lighter in color. The whole was then filtered with suction through a hardened filter paper on a Büchner funnel. The residue, which remained on the Büchner, was transferred to a flask and about 500 c. c. of fresh absolute alcohol was added; the mixture stood at room temperature for 36 hours, the flask being shaken occasionally. Despite the long treatment the alcohol assumed only a light-yellow color. However, when the treatment was continued for six hours more at 55° C. the alcohol assumed a reddish-yellow color, showing that at the higher temperature more of the pigment was extracted by the alcohol. The whole was now filtered through a hardened filter on a Büchner funnel, and since the alcoholic filtrate did not contain any protein, as shown by its failure to give a precipitate on the addition of much water and some sodium chloride, it was discarded. The residue on the Büchner funnel was then ground finely and treated three more times with absolute alcohol at 55° for periods of 24 hours each, when the alcohol failed to take up the pigment to any appreciable degree. The final residue on the Büchner was then extracted with boiling anhydrous ether in a Soxhlet apparatus for five hours, the substance, covered with ether, being allowed to remain in the apparatus overnight. The following morning the

ether, which had assumed a slightly yellow color, was siphoned off by means of the Soxhlet apparatus and discarded. This was followed by two more ether extractions effected in the manner just described, the last ether extract being colorless. The prolamins thus obtained had a grayish-yellow color and weighed 14.3 gm., equivalent to 3.04 per cent calculated on the basis of the oven-dried ryegrass, when dried to practically constant weight during three days in a vacuum over sulphuric acid. This substance, preparation 1, when analyzed was found to contain 4.60 per cent of moisture, 14.16 of nitrogen, and 0.49 of ash, calculated on a basis of the oven-dried prolamins.

TECHNIC USED IN MAKING THREE SUBSEQUENT PREPARATIONS

Another portion of 500 gm. of ryegrass meal was extracted with 2 liters of 70 per cent alcohol at 55° C. for 24 hours, which was followed by the extraction of the filtered residual meal with 1.5 liters of alcohol of the same strength, the operations having been effected as described for preparation 1. The two extracts were united, filtered clear, and concentrated in vacuo, at about 56° to 58°, to two-thirds the original volume, after which the substance was transferred to a porcelain dish and the evaporation continued on the water bath. The supernatant liquid, which was now decanted from the more or less solid protein, was further concentrated in a vacuum and, on cooling, centrifuged. A little more of the protein was deposited on the bottom of the centrifuge tubes. The supernatant liquid of the centrifuge tubes was again concentrated under reduced pressure, yielding another small portion of the protein. Both portions were added to the bulk of the protein, which was now treated with anhydrous ether at room temperature for 36 hours. The ether, which assumed a rich-yellow color, was filtered off and discarded. The solid material which remained on the Büchner funnel was, while still moist, cut up into small pieces, with scissors, and treated with ether for another 24 hours. The ether which had a light-yellow color was filtered off and discarded. This was followed by four treatments of the protein with fresh portions of absolute alcohol at 55° for periods ranging from 24 to 72 hours until the alcohol practically failed to take up coloring matter and the protein completely solidified. Finally the substance was extracted, first with anhydrous ether at room temperature, and then with boiling anhydrous ether in a Soxhlet apparatus for 10 hours. The ether, which appeared almost colorless, was siphoned off and discarded. The residue was preparation 2. From the data given subsequently it will be seen that preparation 2, of which a larger yield was obtained, has a lower nitrogen content than preparation 1. This is undoubtedly due to the fact that the former contains impure protein which was recovered from residual liquids.

A third portion of 500 gm. of ryegrass meal was extracted twice with dilute alcohol as outlined above. The two united and filtered extracts were concentrated under reduced pressure (748 mm. vacuum at 56° to 58° C.). The protein which had separated was purified by repeated treatments with ether, followed by several treatments with absolute alcohol at 55° until the protein had completely solidified. This was again followed by treatments with absolute ether, both at room temperature and at the boiling point of the ether. The prolamins thus purified had a yellow color and were dried in a vacuum over sulphuric acid for 72 hours. This constituted preparation 3.

Still another portion of 500 gm. of ryegrass meal was extracted twice with 70 per cent alcohol at 55° C., with the difference that the first and second extractions lasted 48 to 72 hours, respectively, instead of 24 hours each as in the previous extractions. The separation of the prolamin and its repeated purification, first with anhydrous ether, then with absolute alcohol, and again with anhydrous ether, at room temperature and at the boiling point of ether was effected essentially as outlined in the previous extractions. The final prolamin, on being dried in a vacuum over sulphuric acid for 48 hours, had a grayish-yellow color, and was labeled preparation 4.

The following analytical results were obtained: Preparation 2, which had a yellow color and weighed 18.4 gm. (equivalent to a yield of 3.91 per cent calculated on a basis of the oven-dried ryegrass), had 4.06 per cent moisture, 12.58 per cent nitrogen, and 0.69 per cent ash. Preparation 3, which weighed 14.6 gm. (equivalent to a yield of 3.11 per cent), contained 4.33 per cent of moisture, 14.18 per cent of nitrogen and 0.74 per cent of ash, calculated on a basis of the oven-dried prolamin. Preparation 4, which weighed 13.5 gm. (equivalent to a yield of 2.87 per cent), analyzed as follows: Moisture, 5.29 per cent; nitrogen, 13.96 per cent; ash, 0.83 per cent, calculated on a basis of the water-free protein.

It may be of interest to compare the yields of prolamin obtained from English ryegrass with those from cereal seeds. The data in Table 1 show that the yield of prolamin (provisionally called loliin) from ryegrass was, on the average, higher than the prolamin yield from Canadian and Oregon white winter wheat, but lower than that from South Dakota red spring wheat, and from rye, barley, and maize.

TABLE 1.—Yield of prolamin obtained from ryegrass and cereal seeds

Name of prolamin	Source of prolamin	Yield of prolamin calculated on the basis of—		Reference
		Air-dry seed	Oven-dried seed	
		<i>Per cent</i>	<i>Per cent</i>	
Gliadin.....	Canadian white winter wheat...	2.74	-----	Osborne (24, p. 111).
Do.....	Oregon white winter wheat.....	2.85	-----	Do.
Do.....	South Dakota red spring wheat...	8.15	-----	Do.
Do.....	Rye.....	3.93	-----	Osborne (21, p. 439).
Hordein.....	Barley.....	3.87-4.04	-----	Osborne (22, p. 564).
Zeln.....	Maize.....	5.00	-----	Osborne (23, p. 529).
Loliin, preparation 1.....	English ryegrass.....	2.86	3.04	Jodidi, this paper.
Loliin, preparation 2.....	do.....	3.68	3.91	Do.
Loliin, preparation 3.....	do.....	2.92	3.11	Do.
Loliin, preparation 4.....	do.....	2.70	2.87	Do.

FURTHER PURIFICATION OF THE COMBINED PREPARATIONS

Since the four preparations described above did not differ materially from one another (the range of nitrogen content being from 12.58 to 14.18 per cent and the average 13.72 per cent), it was decided to unite them for the purpose of further purification. The principal impurities recognized were inorganic salts, especially chlorides, and carbohydrates. Therefore, the substance was washed with distilled water on a Büchner funnel provided with a hardened filter paper until the wash water gave but a faint reaction for chlorine, while the

reaction for carbohydrates was also slightly positive. The chocolate-colored residue which remained on the Büchner funnel was minced finely in some absolute alcohol, transferred to a flask to which 1 liter of absolute alcohol was added, and allowed to stand for three days at 55° C. The alcohol assumed a wine-red color. The whole was filtered on a Büchner with suction and washed with absolute alcohol. The residue on the Büchner funnel was again treated with absolute alcohol in the manner just described, after which it was treated with anhydrous ether for several days, the flask being shaken occasionally. On filtering off the ether, the residue, which had a brownish-gray color, was dried in a vacuum over sulphuric acid at room temperature until practically constant weight was obtained. This substance (preparation 5) was analyzed, with the following result: Moisture, 4.88 per cent; nitrogen, 14.30 per cent; ash, 0.80 per cent, calculated to the oven-dried substance.

In order further to purify the protein obtained, the following procedure was adopted: The substance was extracted with 1 liter of 70 per cent alcohol at 57° C. for 24 hours, the flask being shaken occasionally. The brownish-red solution obtained was filtered through a Büchner funnel provided with one soft and one hardened filter paper. There remained on the Büchner funnel a dark-brown quivering jelly with the following properties: When it contained a considerable proportion of water it would quiver as genuine jellies ordinarily do. However, when a part of the water was removed by suction and the jelly became fairly dry in the air overnight it formed a reddish-brown quite elastic cake not unlike rubber. When the jelly became perfectly dry in the air it turned hard and brittle and could be pulverized. It did not stick to either porcelain or paper. The filtered extract was concentrated in a vacuum at 57° to a volume of about 300 c. c. This was poured in a fine stream into 3 liters of ice-cold water. A few cubic centimeters of a concentrated sodium chloride solution was added, which caused a precipitate to appear. The following morning the yellowish-gray precipitate found on the bottom of the beaker was separated from the supernatant liquid by filtration and washed thoroughly with water. The precipitate was now dissolved in 70 per cent alcohol at 57°, a dark wine-red extract resulting. It was filtered clear, concentrated in vacuo to about 250 c. c., and poured in a fine stream into about 2 liters of absolute alcohol. The following morning the precipitated protein was separated from the supernatant liquid by decantation, thrice dehydrated with absolute alcohol, and finally treated with anhydrous ether as already described. The substance (preparation 6) was then separated from the colorless ether by filtration and dried in a vacuum over sulphuric acid for three days.

The dark-brown quivering jelly which had remained on the Büchner funnel from the extract just described was extracted six more times; the concentrated extracts were purified by pouring into large volumes of water and absolute alcohol, respectively, then dehydrated with absolute alcohol, macerated with anhydrous ether, and finally dried in a vacuum over sulphuric acid. These operations were carried out essentially as outlined in describing the first extract. The results obtained are summarized in Table 2.

TABLE 2.—Analyses of the prolamins preparations obtained from extracts 1 to 7

Extract No.	Preparation No.	Yield of prolamin	Moisture ¹	Nitrogen ¹	Ash ¹
		<i>Grams</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1 -----	6	3.3	3.95	14.46	0.44
2 -----	7	4.9	3.91	14.61	.84
3 -----	8	3.5	3.94	14.55	.73
4 -----	9	2.1	3.92	15.02	.99
5 -----	10	2.1	3.90	15.01	.70
6 -----	11	2.1	4.66	15.41	.39
7 -----	12	.9	4.16	15.15	.47

¹ Calculated on the basis of the oven-dried protein.

COAGULATION AND COMPOSITION OF THE PROLAMIN

It became evident from the extractions made that all of the prolamin originally obtained from the English ryegrass meal could not be extracted by digestion with 70 per cent alcohol at 57° C. For this reason different temperatures and different concentrations of alcohol were tried, but with negative results.

The impure prolamin as procured from 2,000 gm. of ryegrass meal was extracted at 55° to 57° with various fresh portions of 70 per cent alcohol successively in the expectation that most if not all of the prolamin would dissolve in that solvent. However, since the various extractions removed but a part of the prolamin, and the quantities obtained, or successive extracts, decreased gradually, it became evident that through the various operations incidental to the purification, especially through the repeated digestions at an elevated temperature, the prolamin acquired the property of becoming difficultly soluble or even insoluble (coagulated). A like behavior was observed by Osborne and by Chittenden and Osborne in their isolation of the prolamins from oats (20) and maize (4, 5).

The preparations thus far obtained were evidently still impure, judging from their comparatively low nitrogen content. This may have been due partly to the fact that in those preparations an endeavor was also made to recover the protein out of the solutions nearly quantitatively. Therefore, it was decided in the next preparations to consider quality only and not to attempt to obtain large yields. The principal change made in procedure consisted in obtaining the next preparations by fractional separation. The analysis of the various preparations so made are summarized in Table 3.

TABLE 3.—Percentage composition of various prolamín preparations (on a moisture and ash free basis)

[illegible]

In reviewing Table 3 it will be seen that there are no essential differences between the soluble and insoluble (coagulated) prolamin preparations as far as their percentage composition is concerned. In other words, through the repeated heatings incidental to purification the prolamin underwent no changes other than to become insoluble. Similar observations were made by Osborne in his work on oats (20), as well as by Chittenden and Osborne in their work on maize (4, 5). In order to have a better idea of the significance of the figures given in Table 3, the average composition of the prolamin from English ryegrass is presented in Table 4, together with the average composition of the prolamins from the various cereals.

TABLE 4.—Average percentage composition of the different prolamins

Item	Zein (from maize)	Kafirin (from kafir)	Hordein (from barley)	Gliadin		Prolamin		
				From wheat	From rye	From oats ¹	From oats ²	From ryegrass
Carbon.....	55.23	55.19	54.29	52.72	52.75	53.08	53.70	53.71
Hydrogen.....	7.26	7.36	6.80	6.86	6.84	6.91	7.00	6.86
Nitrogen.....	16.13	16.44	17.21	17.66	17.72	16.39	15.71	15.80
Sulphur.....	.60	.60	.83	1.14	1.21	2.26	1.76	1.42
Oxygen.....	20.78	20.41	20.87	21.62	21.48	21.36	21.83	22.41
Total.....	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

¹ Average of preparations 7 and 8.² Average of preparations 9, 10, 11, and 12.

THE NAME LOLIIN SUGGESTED FOR THE NEW PROLAMIN

Examination of Table 4 reveals the fact that, with the exception of the prolamins from wheat and rye (gliadin), which are apparently identical, all other prolamins differ more or less from one another in their chemical composition, a fact which has properly led to different individual names for the various prolamins. It further is evident that the prolamin from English ryegrass seemingly differs in its composition from all other prolamins, including gliadin, being nearest to the prolamin from oats, from which it differs, however, in several points. This being the case, the name "loiin" is proposed for this apparently new alcohol-soluble protein. The derivation of this name, from *Lolium perenne*, is fully analogous with that of the names of other well-known prolamins, such as hordein from *Hordeum vulgare* and zein from *Zea mays*. It is quite true that the name loiin has already been proposed for a volatile alkaloid (2, 3) supposedly occurring in the fungus of *Lolium temulentum*. Hofmeister (11), however, has proved that the alkaloid in question does not exist. Instead, he has shown the alkaloid temulin to occur in *L. temulentum*. Therefore, the use of the name loiin appears to be justified and fitting for the newly isolated protein.

NITROGEN DISTRIBUTION IN LOLIIN

For a further characterization of the isolated loiin, a study of its nitrogen distribution was made according to the method of Hausmann (8, 9), as modified by Osborne and Harris (27), and later adapted by Jodidi and Moulton (16). The results obtained are summarized in Table 5. For the sake of comparison the nitrogen distribution of several other prolamins, as reported by Osborne, also is given in the table.

TABLE 5.—Nitrogen distribution of the various prolamins

No.	Prolamin	Amide nitrogen ¹	Humin nitrogen ¹	Diamino nitrogen ¹	Mono-amino nitrogen ¹	Remarks
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
1	Gliadin (from wheat).....	4.33	0.14	0.98	12.21	
2	Hordein (from barley).....	4.01	.23	.77	12.04	
3	Zein (from maize).....	2.97	.16	.49	12.51	
4	Loliin (soluble, from English ryegrass)...	3.32	.58	.50	11.16	Boiled with 20 per cent HCl in a glycerine bath for 12 hours.
5do.....	3.34	.50	.49	10.57	Boiled with 25 per cent H ₂ SO ₄ with Bunsen burner for 24 hours.
6do.....	3.24	.55	.40	11.12	Treated like No. 4.
7	Loliin (insoluble, from English ryegrass)...	3.27	.41	.58	11.00	Treated like No. 5.
8do.....	3.22	.50	.48	10.84	Treated like No. 4.
9do.....	3.24	.50	.66	10.88	Treated like No. 5.

¹ On the basis of the oven-dried prolamin.

It is readily seen in Table 5 that the amide nitrogen content of loliin is lower than that of gliadin and hordein, but higher than that of zein. While the humin nitrogen content of loliin is higher than that of gliadin, hordein, and zein, the reverse is true of the monoamino nitrogen, while the diamino nitrogen of loliin is about equal to that of zein but lower than that of gliadin and hordein. When preparations Nos. 4 to 6, representing the soluble modification of loliin, are compared with Nos. 7 to 9, representing the insoluble modification, it will be seen that the nitrogen distribution in both is practically identical. This shows, as in the case of their identical ultimate composition, that loliin, through repeated heating incidental to its purification, underwent no chemical changes. It may be mentioned here that the ryegrass used was not quite free from chaff, and, despite considerable efforts, it has been impossible to obtain loliin in a state of perfect purity, the final preparations always containing some coloring matter and perhaps other impurities. The next step to be undertaken by this laboratory will be the preparation of the highly purified protein in large quantity in order that it may be hydrolyzed into the individual amino acids of which it is composed.

SUMMARY

The alcohol-soluble protein occurring in English ryegrass was isolated for the first time, and its purification, analysis, and properties are described. It has been named loliin, the derivation of this name being analogous to that of the names of other well-known prolamins, such as hordein and zein. During repeated alcoholic treatments at elevated temperatures, incidental to the purification, a considerable portion of the prolamin becomes insoluble. The soluble and insoluble portions of the prolamin do not appear, however, to differ from each other chemically, since they have practically the same ultimate composition as well as the same nitrogen distribution in their respective molecules.

LITERATURE CITED

- (1) ARNAUD.
1889. RECHERCHES SUR LA CAROTINE; SON RÔLE PHYSIOLOGIQUE PROBABLE DANS LA FEUILLE. *Compt. Rend. Acad. Sci. [Paris]* 109: 911-914, illus.
- (2) BLEY, L. F.
1834. UEBER DEN GIFTSTOFF UND DIE BESTANDTHEILE DES TAUMELLOLCHS. *LOLIUM TEMULENTUM* L. *Repertorium Pharm. [Nürnberg]* 48: [169]-200.
- (3) ———
1837. EINIGE VERSUCHE ZUR VERVOLLSTÄNDIGUNG DER CHEMISCHEN UNTERSUCHUNG DES TAUMELLOLCHSAMENS. *Repertorium Pharm. [Nürnberg]* (2 Reihe) 12: 175-180.
- (4) CHITTENDEN, R. H., and OSBORNE, T. B.
1891. A STUDY OF THE PROTEIDS OF THE CORN OR MAIZE KERNEL. *Amer. Chem. Jour.* 13: 453-468, 529-552.
- (5) ——— and OSBORNE, T. B.
1892. A STUDY OF THE PROTEIDS OF THE CORN OR MAIZE KERNEL. II. PROTEIDS SOLUBLE BOTH IN WATER AND IN DILUTE SALT SOLUTIONS. *Amer. Chem. Jour.* 14: 20-44.
- (6) DEETZ, R.
1873. UNTERSUCHUNGEN VON *LOLIUM PERENNE* IN VERSCHIEDENEN STADIEN DER ENTWICKLUNG. *Jour. Landw.* 21: 57-88.
- (7) GAUTIER, A.
1895. SUR LA PLURALITÉ DES CHLOROPHYLLES. REMARQUES À PROPOS DE LA NOTE DE M. ÉTARD. *Compt. Rend. Acad. Sci. [Paris]* 120: 355-356.
- (8) HAUSMANN, W.
1899. UEBER DIE VERTHEILUNG DES STICKSTOFFS IM EIWESSMOLEKÜL. *Ztschr. Physiol. Chem.* 27: [95]-108.
- (9) ———
1900. UEBER DIE VERTHEILUNG DES STICKSTOFFS IN EIWESSMOLEKÜL. II. MITTHEILUNG. *Ztschr. Physiol. Chem.* 29: 136-145.
- (10) HOFFMAN, W. F.
1925. AN ALCOHOL-SOLUBLE PROTEIN ISOLATED FROM POLISHED RICE. *Jour. Biol. Chem.* 66: 501-504.
- (11) HOFMEISTER, F.
1892. DIE WIRKSAMEN BESTANDTHEILE DES TAUMELLOLCHS. *Arch. Expt. Path. u. Pharmacol.* 30: 202-230.
- (12) JODIDI, S. L.
1924. PHYSIOLOGICAL STUDIES ON CEREALS. II. THE OCCURRENCE OF AMINO ACIDS AND POLYPEPTIDES IN THE UNGERMINATED OAT KERNEL. *Jour. Franklin Inst.* 198: 201-211.
- (13) ———
1925. PHYSIOLOGICAL STUDIES ON CEREALS. III. THE OCCURRENCE OF POLYPEPTIDES AND AMINO ACIDS IN THE UNGERMINATED MAIZE KERNEL. *Jour. Agr. Research* 30: 587-592.
- (14) ———
1927. THE NITROGEN COMPOUNDS OF THE RICE KERNEL AS COMPARED WITH THOSE OF OTHER CEREALS. *Jour. Agr. Research* 34: 309-325.
- (15) ——— and MARKLEY, K. S.
1923. THE OCCURRENCE OF POLYPEPTIDES AND FREE AMINO ACIDS IN THE UNGERMINATED WHEAT KERNEL. *Jour. Amer. Chem. Soc.* 45: 2137-2144.
- (16) ——— and MOULTON, S. C.
1919. THE CAUSE OF AND REMEDY FOR CERTAIN INACCURACIES IN HAUSMANN'S NITROGEN DISTRIBUTION METHOD. *Jour. Amer. Chem. Soc.* 41: 1526-1531.
- (17) ——— and PEKLO, J.
1929. SYMBIOTIC FUNGI OF CEREAL SEEDS AND THEIR RELATION TO CEREAL PROTEINS. *Jour. Agr. Research* 38: 69-91.
- (18) ——— and WANGLER, J. G.
1925. PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON CEREALS. IV. ON THE PRESENCE OF AMINO ACIDS AND POLYPEPTIDES IN THE UNGERMINATED RYE KERNEL. *Jour. Agr. Research* 30: 989-994.

-
- (19) JOHNS, C. O., and BREWSTER, J. F.
1916. KAFIRIN, AN ALCOHOL-SOLUBLE PROTEIN FROM KAFIR, ANDROPOGON SORGHUM. *Jour. Biol. Chem.* 28: 59-65.
- (20) OSBORNE, T. B.
1891-92. THE PROTEIDS OR ALBUMINOIDS OF THE OAT-KERNEL. *Amer. Chem. Jour.* 13: 327-347, 385-413; 14: 212-224.
- (21) ———
1895. THE PROTEIDS OF THE RYE KERNEL. *Jour. Amer. Chem. Soc.* 17: [429]-448.
- (22) ———
1895. THE PROTEIDS OF BARLEY. *Jour. Amer. Chem. Soc.* 17: 539-567.
- (23) ———
1897. THE AMOUNT AND PROPERTIES OF THE PROTEIDS OF THE MAIZE KERNEL. *Jour. Amer. Chem. Soc.* 19: 525-532.
- (24) ———
1907. THE PROTEINS OF THE WHEAT KERNEL. 119 p. Washington, D. C. (Carnegie Inst. Wash. Pub. 84.)
- (25) ———
1910. EIWEISSTOFFE. A. DARSTELLUNG DER PROTEINE DER PFLANZENWELT. In Aberhalden, E., *Handbuch der biochemischen Arbeitsmethoden* Bd. 2, p. [270]-334, illus.
- (26) ———
1924. THE VEGETABLE PROTEINS. Ed. 2, 154 p., illus. London, New York, [etc.].
- (27) ——— and HARRIS, I. F.
1903. NITROGEN IN PROTEIN BODIES. *Jour. Amer. Chem. Soc.* 25: [323]-353.
- (28) PEKLO, J.
1913. ÜBER DIE ZUSAMMENSETZUNG DER SOGENANTEN ALEURONSCHICHT. *Ber. Deut. Bot. Gesell.* 31: 370-384, illus.
- (29) RITTHAUSEN, H.
1872. DIE EIWEISSKÖRPER DER GETREIDEARTEN, HÜLSENFRÜCHTE UND ÖLSAMEN. 252 p. Bonn.
- (30) SCHULZE, E., STEIGER, E., and BOSSHARD, E.
1887. UNTERSUCHUNGEN ÜBER DIE STICKSTOFFHALTIGEN BESTANDTHEILE EINIGER RAUHFUTTERSTOFFE. *Land. Vers. Sta.* 33: [89]-123.
- (31) STUTZER, A.
1880. UNTERSUCHUNGEN ÜBER DIE QUANTITATIVE BESTIMMUNG DES PROTEINSTICKSTOFFS UND DIE TRENNUNG DER PROTEINSTOFFE VON ANDEREN IN PFLANZEN VORKOMMENDEN STICKSTOFFVERBINDUNGEN. *Jour. Landw.* 28: 103-123.

THRESHER INJURY A CAUSE OF BALDHEAD IN BEANS¹

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INTRODUCTION

Baldhead of beans (*Phaseolus vulgaris* L.), sometimes called snake-head, for a number of years has attracted the attention of seed growers and farmers, who have observed on seedlings the partial or entire absence of the primary leaves and the growing tip. Such plants, remaining dwarfed, are soon completely hidden by the foliage of the surrounding plants, and the erroneous impression is often gained from later inspections of the field that the plants have recovered and developed normally. Concern would not be aroused about the disease until similar seedlings were observed in a later planting or during another season.

Little information is available as to the history of baldhead, although interviews with seedsmen and growers have revealed the fact that it has been known for a number of years and is apparently on the increase. Because of the economic importance of baldhead and numerous inquiries as to its control, investigations were undertaken to determine its cause. What appeared a priori to be a simple problem proved in the end to be somewhat difficult and complicated. At first thought one might reasonably conclude that this abnormality is caused by insects, fungi, or bacteria; but certain relationships of baldhead to farm operations and its appearance in disease-free seed suggested that it could not be attributed to these agencies alone.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Inspection of bean fields in various parts of the United States revealed the fact that baldhead occurs practically wherever the crop is grown. The loss from baldhead plants, which make a poor growth and during the entire season produce at best only one or two imperfectly filled pods, is about equivalent to the percentage of affected plants at germination. This percentage varies according to the variety, the method of handling the crop, and the season. As a matter of fact, the amount of baldhead of any susceptible variety may vary from one season to the next as much as 5 to 10 per cent. Some varieties, such as Bountiful,³ Refugee, and Improved Kidney Wax, are very susceptible, percentages of 10 to 30 not being uncommon.

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³ The variety names used in this paper are those employed by the trade.

SYMPTOMS

Baldhead is caused by three distinct agencies, each producing well-defined characteristics. One type, which is by far the most common, is, as will be shown later in this paper, the result of mechanical injury. (Fig. 1, A-D.) The second type is caused by bacteria (fig. 1, E-G), and the third by insects. Although baldhead caused by insects has been investigated by Hawley⁴ and has received no attention during these studies, it will be discussed briefly elsewhere.

Mechanical injury and bacterial infection produce quite different symptoms. In the case of mechanical injury the plumule may be entirely absent (fig. 1, B-D) or only vestiges of it remain. The plumule may at times remain attached to the epicotyl (fig. 1, A), although it is fractured just beneath the primary leaves, thus rendering further development impossible. These symptoms stand in striking contrast to those resulting from bacterial attack, in which case the entire plumule may be more or less completely destroyed (fig. 1, G) or the primary leaves may be badly mutilated, the terminal bud often being destroyed by the organism so that only remnants of the vascular foliar tissue remain. (Fig. 1, E, F.) Sometimes, in the absence of the terminal bud, the epicotyl elongates, often attaining a length of one-eighth (fig. 1, B, D) to three-fourths (fig. 1, A, C) of an inch. At this stage the plant may die, but more often buds develop (fig. 1, A, B) in the axils of the cotyledons, resulting in a compact growth of several leaves and branches with short internodes.

EXPERIMENTAL PROCEDURE

MATERIAL STUDIED

No investigations have been made for the single purpose of determining the entire range of plants subject to baldhead. Reports have been received of its occurrence on the different field and snap varieties of *Phaseolus vulgaris* and on *P. lunatus* L., the two species to which the investigations have been largely restricted. These studies were originally undertaken for the purpose of determining the cause of baldhead, and in so doing a number of different varieties, but by no means all, have been employed. Snap-bean varieties appeared to be especially subject to the disease, and for that reason most of the investigations were limited to them, a few varieties of the field type being used for comparison. From a more extended study of the different varieties it was found that field beans, as a group, are more resistant than the snap-bean varieties. Lima beans were not at first brought within the scope of the investigation, but a single germination test of one variety demonstrated a percentage of baldhead about equivalent to the snap-bean average. In view of this fact, three other varieties of Lima beans were brought under observation, as well as the Blackeye cowpea (*Vigna sinensis* Endl.) and the tepary bean (*P. acutifolius* var. *latifolius* G. F. Freeman).

GERMINATION

Preliminary experiments indicated that the presence of baldhead could be determined at about the time when the primary leaves of

⁴ HAWLEY, I. M. INSECTS AND OTHER ANIMAL PESTS INJURIOUS TO FIELD BEANS IN NEW YORK. N. Y. (Cornell) Agr. Sta. Mem. 55, p. 945-1037, illus. 1922.

normal plants had attained a length about equal to that of the cotyledons. Beans germinated well in the several different materials tried, such as sand, sphagnum, soil, and between blotting papers.

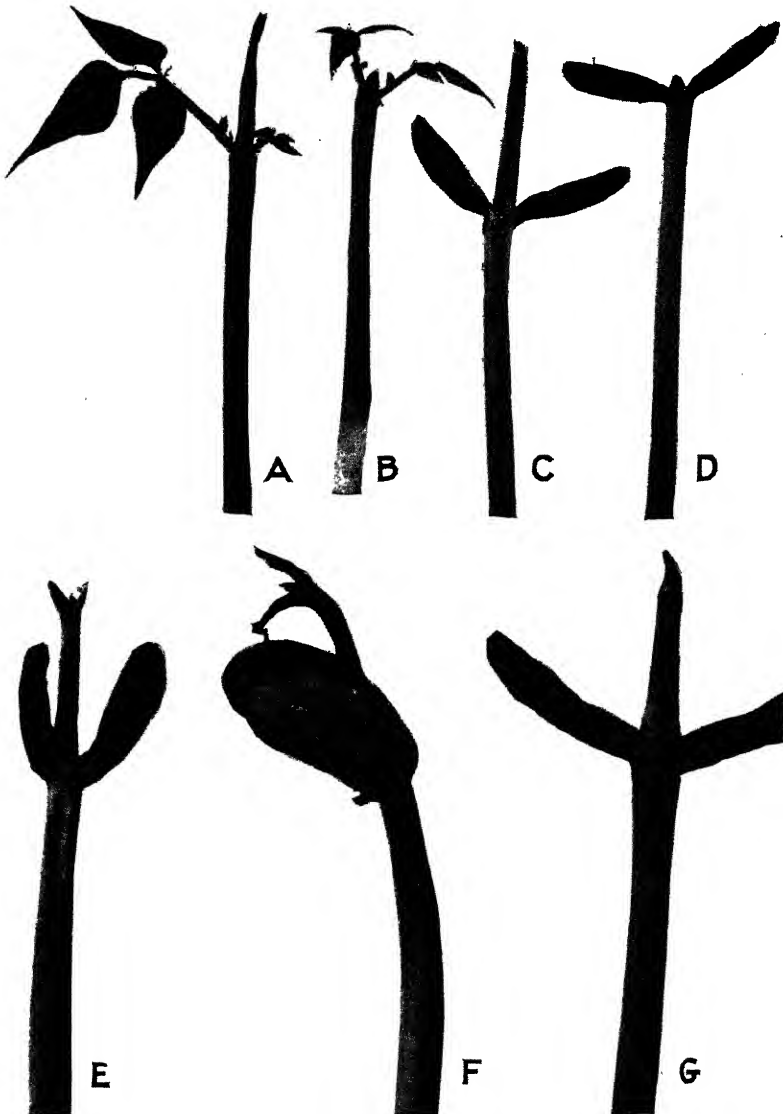


FIGURE 1.—Baldhead of beans as a result of: A-D, mechanical injury, which is characterized by the entire absence or only the vestiges of a plumule; E-G, injury caused by parasitic organisms, such as *Bacterium phaseoli*, which either destroy the entire plumule (G) or partially mutilate the primary leaves, leaving only ragged vestiges of the foliar tissue (E, F). E, F, G, slightly enlarged

The use of soil, sand, and sphagnum was objectionable because they were bulky or heavy and had to be sterilized each time before being used. Furthermore, they had no advantages over the following rapid and reliable method which was finally employed.

One hundred seeds, selected to insure apparent freedom from parasitic organisms, were disinfected for three minutes in a 1-1,000 solution of mercuric chloride and immediately rinsed in sterile water. The tests were conducted in moist chambers kept at laboratory temperature (22°-28° C.). In each moist chamber was placed a wire support, made of a square piece of heavy 4-mesh wire screen with the corners turned down about 1 inch; on this, blotting paper of proper size was laid. The seeds were then placed in the chamber so that they would not touch one another, and a second piece of blotting paper of the same size as the first was placed on top of them, after which all was thoroughly soaked with water. About one-fourth to one-half inch of water was poured into the moist chambers to maintain a relatively high humidity. Under these conditions most varieties germinated rather quickly, and at the end of five or six days radicles an inch or more in length had developed. At this stage the blotting papers were removed and the germinating beans were placed directly on the wire screen with the radicles inserted through the meshes and extending into the liquid below. Because of the paucity of food material in sterile distilled water, it was often replaced at this time by an equivalent quantity of Knop's solution. The presence of baldhead can be determined very effectively at this stage of development, but the seedlings were usually allowed to grow a few days longer until the primary leaves were one-fourth to one-half inch or even more in length, when a count was made of the affected plants.

RESULTS

EFFECT OF SEED MATURITY

In certain seed-growing districts of the West the season is comparatively short, so that varieties requiring a long season are sometimes frosted before all the seeds are mature—a contingency that was considered as having a possible causal relation to baldhead. With this in mind, some pods were gathered from plants in Virginia and in Colorado before the seeds were mature, and again from the same plants after the seeds had fully ripened. Although some of the immature seeds were badly shriveled, they germinated in most cases. The germination tests were made under similar conditions, with the result that there was no baldhead in either mature or immature seeds, showing that the stage of maturity is probably in no way associated with the malady.

INHERITABILITY

In 1926 seeds were collected from baldhead plants and threshed by hand, one part being tested for baldhead by germinating between blotting paper and the other planted in the field the following spring. No baldhead plants developed from any of these seeds. Second-generation seeds were also normal.

DESICCATION

Desiccation of the seeds, such as probably occurs in the arid regions of the West, resulting in injury to the plumule or epicotyl, was suggested as a possible cause of baldhead. An experiment was therefore designed to show whether such a relation existed. The varieties employed (Bountiful, Black Wax, and Kentucky Wonder Wax) contained a known percentage of baldhead and had been grown in Idaho and threshed by machine. Bountiful seeds, grown in Virginia,

threshed by hand, and containing no baldhead, were used for comparison. Desiccators containing concentrations of sulphuric acid previously calculated to give relative humidities of 1.5, 10.5, 21.5, 33, and 45 per cent were used. At the end of each month for four consecutive months 100 seeds of each lot were removed from each of the desiccators and germinated. The details of the results will not be given, inasmuch as the very slight increase in baldhead in those lots exposed to low relative humidities fell easily within the range of experimental error. The Kentucky Wonder Wax and Bountiful varieties, originally containing no baldhead, might, after an exposure to desiccation for four months, show 1 or 2 per cent of baldhead. From these results it seems that desiccation alone has very little if any effect on the production of baldhead.

MACHINE-THRESHED COMPARED WITH HAND-THRESHED BEANS

SNAP AND FIELD BEANS

Germination tests of beans grown in California, Idaho, and Colorado, in comparison with seeds grown at the Arlington Experiment Farm, Rosslyn, Va., revealed some striking differences. The eastern seeds were harvested by hand and threshed either by hand or by pounding with a stick and contained little or no baldhead, whereas the seeds from the West contained a considerable amount. In seeking for an explanation of this difference, the probable variations in methods of handling were considered. The western seeds, obtained from commercial sources, were presumed to have been threshed by machine. This presumption gave a clue to the possible cause and suggested the desirability of a series of comparative germination tests. The earlier investigations were followed by similar tests in which the seeds were collected in such a way as to yield a direct comparison between machine-threshed and hand-threshed seeds. A number of varieties known to be subject to baldhead were chosen for study, and the seeds were picked and threshed by hand. Seeds from the same crop were obtained after machine threshing, and both were subjected to comparative germination tests. Hand-picked and hand-threshed seeds were also compared with seeds threshed by large and small threshers and by a flail.

The conclusions are drawn largely from the results of laboratory germination tests, but in 1927 and 1928 plantings were made from the same lots of seeds in the field in Colorado and in Virginia with the result that those varieties that gave a high percentage of baldhead in the laboratory generally gave a correspondingly high percentage when planted in the field. As might be expected, there was some variation due to the fact that insects and predatory animals, such as rodents, birds, etc., were responsible for the destruction of some plants and plant parts in the field. The evidence indicates, however, that the laboratory studies are a reliable guide as to what can be expected under field conditions.

The results given in Table 1, which includes all those varieties where a direct comparison of different methods of threshing could be made, show that threshing with either a large or a small machine in some cases produces a high percentage of baldhead. Flailing out the seeds causes some damage, but considerably less than either type of

machine threshing, while there were only two baldhead beans in all the hand-shelled beans tested.

TABLE 1.—Variety, source of seed, and influence of method of threshing on the percentage of baldhead in beans

Variety	Source	Percentage of baldhead resulting from threshing by—			
		Large machine	Small machine	Hand	Flail
Bountiful.....	Colorado.....	6.2	0
Burpee's Stringless Green Pod.....	do.....	4	0
Full Measure.....	do.....	13	1	0
Improved Kidney Wax.....	do.....	8.2	6	0
Stringless Green Refugee.....	do.....	19.2	0
Davis White Wax.....	Idaho.....	27.8	0
Full Measure.....	do.....	14.7	1
Giant Stringless Green Pod.....	do.....	10.4	1
Improved Kidney Wax.....	do.....
Burpee's Stringless Green Pod.....	Wisconsin.....	18.5	0
Currie's Rust Proof.....	do.....	20	1
Early Stringless Refugee.....	do.....	23.3	5.5
Giant Stringless Green Pod.....	do.....	22.7	1.1
Hodson Wax.....	do.....	14.4	0
Improved Kidney Wax.....	do.....	20.6	3.6
Round Pod Kidney Wax.....	do.....	21	2.3
Sure Crop Wax.....	do.....	4	0
Wardwell's Wax.....	do.....	12.7	0

For a comparative study of the percentage of baldhead in different lots of the same varieties of snap beans obtained from different sources, seeds were secured which were in most cases machine threshed. In a few lots shelling was done by hand; in others the information was not available, because the seeds were obtained from wholesale houses and from growers, but they are assumed to have been threshed by machine.

Table 2 shows that baldhead occurs in susceptible varieties of beans regardless of their source, if they are threshed by machine. Considerable variation occurs in seeds from different sources (Table 3), which is due probably not so much to any influence of the place of origin as to the condition of the pods when threshed. Some evidence has been collected which suggests that, if the vines and pods are damp when threshed the amount of baldhead is likely to be less.

LIMA BEANS

Like the snap and dry-shell types, Lima beans have been observed in some cases to develop a considerable percentage of baldhead in commercial field plantings, the fatalities often being so high as to arouse considerable concern on the part of growers and seedsmen. Only four varieties of Lima beans have been tested for baldhead, one of which (Henderson Bush Lima) was obtained from a seedsman in California and is definitely known to have been grown there. Three other varieties known to the trade as Fordhook Bush Lima, Emerald Isle Pole, and Sieva were purchased in Washington, D. C., from a seed dealer who had no information as to where the seed was grown or how it was threshed. It is not unlikely that they also came from California since most of the Lima-bean seed produced in this country is grown along the southern coast of California, and it is reasonably safe to assume that they were machine threshed.

TABLE 2.—Percentage of baldhead in beans obtained from different sources when threshed by different methods

Variety	Source	Percentage of baldhead resulting from threshing by—		
		Machine	Hand	Unknown method
Late Refugee.....	Idaho.....	18.5		
	Colorado.....	9.6		
	Virginia.....		0	
Refugee, 1000-1.....	Michigan.....			0
	do.....			5.1
Early Refugee.....	do.....			10.6
	Idaho.....	23.8		
	Colorado.....	7.4		
Bountiful.....	Virginia.....		0	
	Michigan.....			0
	do.....			2
	do.....			5
	Idaho.....	19.1		
	Colorado.....	14		
Red Valentine.....	Virginia.....		0	
	Michigan.....			5.5
	do.....			6
	do.....			4
	Idaho.....	15		
Black Valentine.....	Colorado.....	10.6		
	Virginia.....		0	
Kentucky Wonder Wax.....	Idaho.....	1.1		
	California.....	3		
Longfellow.....	Idaho.....	19.6		
	Virginia.....		0	
Black Wax.....	Idaho.....	22.1		
Black Wax Pencil Pod.....	California.....	8		
Prolific Black Wax.....	Colorado.....	9.4		
Great Northern.....	Idaho.....	1.1		
	do.....			0
Perry Marrow.....	New York.....			0
Well's Red Kidney.....	do.....			0
Michigan Pea Bean.....	Michigan.....			2
Robust.....	do.....			2
Improved Kidney Wax.....	Wisconsin.....	24.5		
Red Kidney.....	California.....	25.5		
Bayo.....	do.....	11		
Cranberry.....	do.....	2		
California Pink.....	do.....	1		
Large White.....	do.....	8.5		
Genuine Small White.....	do.....	1.1		
Blue Pod Small White.....	do.....	2		
California Red.....	do.....	1.2		
Dutch Caseknife.....	do.....	18.9		
Lazy Wife.....	do.....	2.1		
Striped Creaseback.....	do.....	3		
Golden Cluster Wax.....	do.....	5.4		
King Mammoth Horticultural.....	do.....	12.2		
French Horticultural.....	Idaho.....	21.1		
Dwarf Horticultural.....	do.....	17.4		
Tennessee Green Pod.....	do.....	2.1		
Currie's Rust Proof.....	do.....	15.8		
Champion Bush.....	do.....	2		
Pinto.....	Colorado.....	1.5		
Red Mexican.....	Arizona.....			2.1

* Average of 6 lots, varying from 19 to 30 per cent baldhead.

TABLE 3.—Summary of the percentage of baldhead in all varieties of beans from different sources when threshed by the different methods

Source of seed	Number of seeds	Percentage of baldhead resulting from threshing by—		
		Machine	Flail	Hand
All sources.....	1,874			0.1
California.....	2,140	9.1		
Colorado.....	858	8.9		
Idaho.....	4,910	12.2		
Virginia.....	2,628		2.2	
Wisconsin.....	595	24.7		
Do.....	900	* 17.8		

* Threshed by small machine.

In germination tests similar to those conducted with the varieties of *Phaseolus vulgaris*, the following percentages of baldhead were obtained: Sieva, 2.17; Henderson Bush Lima, 11.11; Emerald Isle Pole, 13.67; and Fordhook Bush Lima, 15.95. These data show that Lima beans are liable to baldhead in percentages sufficiently large to result in considerable reduction in yield.

COWPEAS AND TEPARY BEANS

There was no baldhead in the Blackeye cowpeas tested. In the tepary bean there was 6.88 per cent of baldhead.

HISTOLOGY

An examination of the dry embryo or of the seed in a very early stage of germination, even before the integuments are ruptured, frequently reveals a fracture

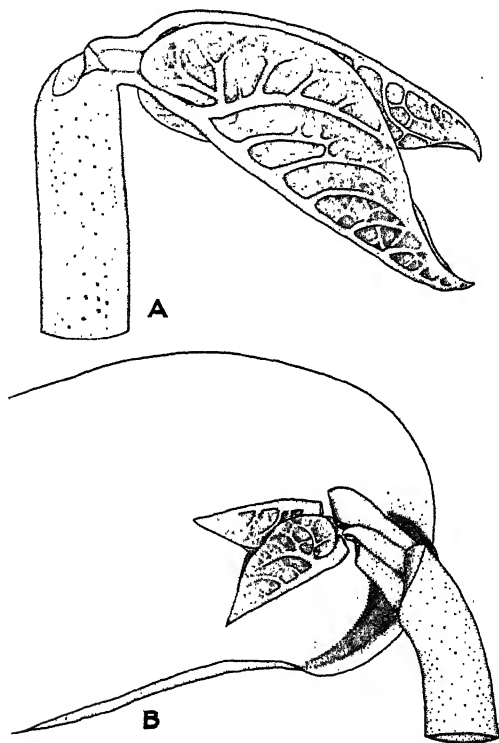


FIGURE 2.—Two bean embryos several days after germination: A, Normal embryo with both cotyledons removed; B, baldhead bean with one cotyledon removed to show the plumule broken off from the epicotyl. $\times 5$

of the epicotyl just below the plumule, in which the latter may be partially or completely broken from the epicotyl. Figure 2, B, shows an early stage of germination of a seed in which the plumule is completely detached from the epicotyl. This should be compared with Figure 2, A, a normal seed germinated at the same time. Fractures such as those shown in Figure 2, B, are easy to detect by the unaided eye, but results indicated that baldhead sometimes occurs when the plumule and epicotyl appear to be normal, which suggested the possibility that an invisible injury to the tissues might occur. Consequently, permanent microscopic sections were made of normal and baldhead material. The seeds were germinated for a few days between blotting paper, and when the radicle had

elongated slightly the cotyledons were carefully removed and the epicotyl with a portion of the hypocotyl was fixed in Carnoy's or formol-acetic-alcohol solution. After this they were carried through the usual processes of embedding, sectioning, staining, and mounting.

From among the many sections showing some type of injury, one was selected for the purpose of illustration. Figure 3, B, gives in outline a portion of this section, including a part of the two primary

leaves (*c*), the terminal bud (*d*), and the region just below the plumule, included between the two dotted parallel lines. A cursory examination of this region shows that the tissue of the epicotyl just between the primary leaves is torn at both margins, the tear extending slightly in the direction of the terminal bud and toward the center (fig. 3, B, *a*), partially dismembering the two primary leaves and terminal bud from the remainder of the epicotyl. Sometimes the tissue in the region below the terminal bud (fig. 3, B, *b*) is torn in one or more places, resulting in the production of irregularly shaped cavities, which vary in extent and number but which are usually in the general location shown by *b*. Figure 3, A, is a photomicrograph of the region shown between the dotted lines of Figure 3, B, and is of about the same magnification, while Figure 3, C, is a detailed drawing of the arrangement of the cells of this region at a like magnification.

The fractures in the epicotyledonary region differ somewhat in general appearance, but all produce the same result—the plumule fails to develop and finally becomes detached from the rest of the epicotyl. Sometimes the tear extends from the margin almost to the center of the epicotyl, and the plumule is held in place by only a few rows of cells.

RELATION OF DIAMETER OF EPICOTYL TO VARIETAL SUSCEPTIBILITY

An examination of the embryo of an ungerminated bean seed (fig. 4) with a dissecting microscope revealed a considerable diminution in the diameter of the epicotyl just below the plumule, where most of the fractures leading to the production of baldhead plants take place. This fact suggested the possibility that there might be some relationship between the diameter of the epicotyl and susceptibility to baldhead, those embryos with a small diameter in the epicotyledonary region presumably being more liable to injury from the threshing machine than those with larger epicotyls. Measurements then were made through the smallest diameter of the epicotyl of 25 seeds each of 18 varieties, which were selected to include 6 each of those which would be classed as very susceptible, susceptible, and highly resistant. Those varieties in which there was no baldhead, or not more than 1 or 2 per cent, were classified as immune or resistant. The embryo was carefully removed from the cotyledons, and measurements were made at a magnification of approximately 57 diameters, with a camera lucida being used to project the images upon a millimeter rule on the top of a table.

While one may not be justified in attaching any great importance to these data, the results show that the epicotyledonary diameter of the very susceptible, susceptible, and highly resistant groups average 0.45, 0.49, and 0.55 mm., respectively, the maximum being 22.2 per cent greater than the minimum. Of course it does not necessarily follow that the epicotyl with a small diameter is more easily broken than one with a large diameter, although, everything else being equal, such would probably be the case. The fact remains, at least, that the highest percentage of baldhead was found to occur in those varieties with the smallest diameter of the epicotyl.

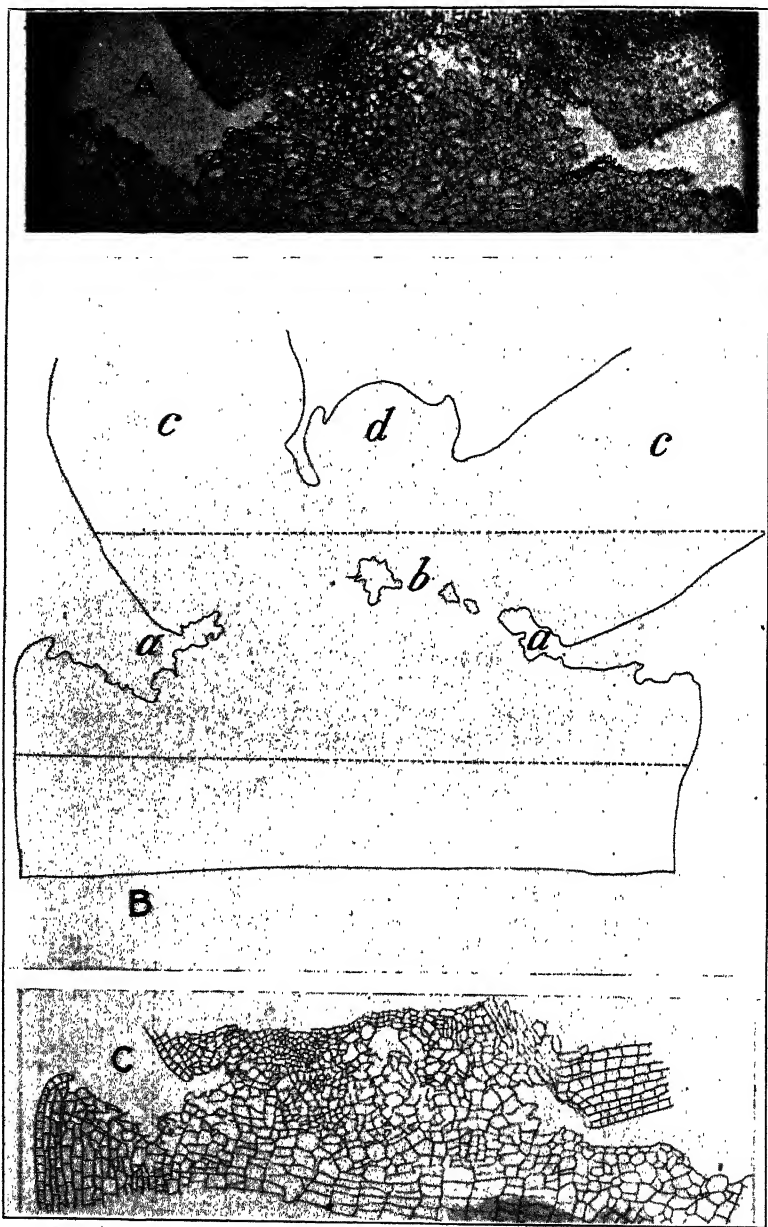


FIGURE 3.—Histology of baldhead: A, Photomicrograph of the area of a baldhead bean represented in B between the dotted lines; B, outline drawing, showing the primary leaves (c), the terminal bud (d), and the region where fractures occur (a, b); C, camera-lucida drawing, showing fracture and arrangement of cells in region covered by A. All about $\times 70$

DISCUSSION

RELATION OF INSECTS TO BALDHEAD

According to Hawley,^{5,6} the seed-corn maggot (*Phorbia fusciceps* Zett.) causes from 50 to 75 per cent damage to beans in New York and other States as a result of abscising the plumule, thus causing the plants to develop baldhead, or tunneling into the cotyledons while the seed is still in the ground. The insect also attacks the stem beneath the ground. This led Hawley to conclude that the entire loss attributed to the maggot was not due solely to the injury to the plumule, but in part to the injury to the stem.

BACTERIA AND FUNGI AS CAUSAL FACTORS IN BALDHEAD

While agreeing with Hawley⁷ that the seed-corn maggot is responsible for most of the baldhead, Burkholder⁸ claimed that *Bacterium*

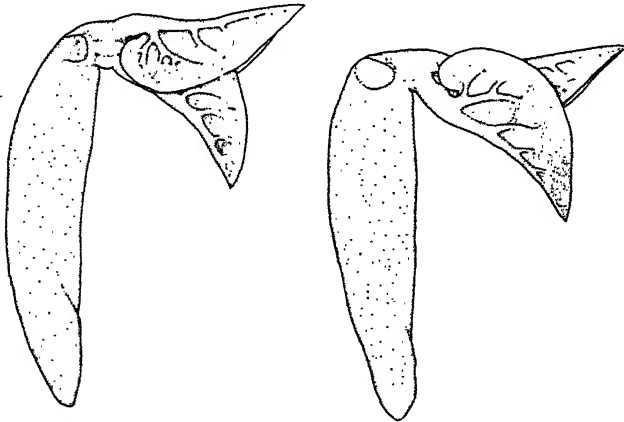


FIGURE 4.—Two embryos from dry beans, showing constriction in the epicotyl at the region where fractures caused by the threshing machine take place. X 5

phaseoli EFS., as well as *Thielavia basicola* Zopf and a species of *Rhizoctonia*, may cause the same type of injury, although he gave no evidence to show that he produced the disease by inoculation with any of these organisms. His conclusions appear to be drawn entirely from the fact that *Bact. phaseoli*, *T. basicola*, and *Rhizoctonia* were isolated from baldhead plants. As additional proof Burkholder cited the results of planting blighted bean seeds in the greenhouse, 20 per cent of one lot producing baldhead while other plantings gave varying percentages.

In this connection, attention should be called to the fact that a high percentage of baldhead may occur in seed produced in regions of the West where bacterial blight does not occur, and attempts to isolate bacteria from specimens from those regions have always resulted in failure. Furthermore, it has been the writer's experience that in

⁵ HAWLEY, I. M. Op. cit. (See footnote 4.)

⁶ HAWLEY, I. M. SOME NOTES ON PHORBIA FUSCICEPS AS A BEAN PEST. Jour. Econ. Ent. 12: 203-205, illus. 1919.

⁷ HAWLEY, I. M. Op. cit. (See footnote 6.)

⁸ BURKHOLDER, W. H. THE BACTERIAL BLIGHT OF THE BEAN: A SYSTEMIC DISEASE. Phytopathology 11: [61]-69. 1921.

many cases isolations from the tips of baldhead plants from badly blighted seeds yield neither the blight nor the wilt organisms.

While the conclusions of Hawley and Burkholder are not questioned, the seed-corn maggot, *Bacterium phaseoli*, *Thielavia basicola*, and *Rhizoctonia* are not the sole causes of baldhead. The association of an organism, such as *Bact. phaseoli*, with baldhead does not constitute complete proof of a causal relationship. The bean-blight organism may gain entrance to the seed through the micropyle,⁹ so that when germination starts, infection on the inner faces of the cotyledons is not uncommon. By the time the seedling has emerged from the soil the denuded epicotyl of already injured seeds may have attained a length varying from one-eighth to three-fourths of an inch; it is quite probable that it might have come in contact with blight lesions of the cotyledons and become infected or that it acts as a carrier only.

MECHANICAL INJURY DUE TO CONDITION OF THE SOIL

Many growers attribute baldhead to a mechanical injury of the epicotyl as the seedling pushes through the soil. Such accidents are supposed to be greatly increased when the penetration of the soil by the seedling is made more difficult by a dry, hard crust. However, it is not the epicotyl, protected as it is by the cotyledons, which is broken, but the hypocotyl, so that no further development takes place. This injury is sometimes mistaken for baldhead.

THRESHER INJURY

The occurrence of baldhead in bean seeds free from pathogenic organisms and in seeds germinated in the laboratory under conditions precluding infection by parasites was convincing evidence that there was another cause quite apart from insects, bacteria, and fungi. In seeds of almost every snap-bean variety grown in the West a considerable percentage of baldhead was found. This suggested a relationship between western-grown seed and the malady, but the results of investigations showed that there was nothing inherent in western-grown seed that would render it any more susceptible to baldhead than seed grown elsewhere. Commercial bean seed is raised in a comparatively few regions, but in all cases it is handled in the same manner. In comparing the methods of harvesting seed the commercial method of machine threshing is the only essential difference, and here apparently lies the causal factor to which is attributed more baldhead than to all the other factors combined—injury to the epicotyl while the seeds are being threshed.

The germination of hundreds of bean seeds showed that baldhead occurs in certain varieties threshed by machine, while seeds of the same lot shelled by hand gave none on germination by the same method. A microscopic examination of permanently mounted material showed that a fracture of the epicotyl of beans destined to become affected with baldhead occurred just beneath the plumule in machine-threshed seed. However, inasmuch as no such wound is found in hand-shelled beans, the evidence seems quite conclusive that the break is caused by the beans being violently struck by the teeth of the thresher cylinder or hurled against the teeth of the concave during threshing. Some varie-

⁹ ZAUMMEYER, W. J. SEED INFECTION BY BACTERIUM PHASEOLI. (Abstract) Phytopathology 19: 96. 1929.

ties are difficult to shell and can only be threshed by speeding up the cylinders, which might reasonably be expected to result in the splitting of many seeds and the fracturing of the epicotyl of others. The degree of desiccation of the seed at the time of threshing has been suggested as being somewhat correlated with baldhead; that is, if the beans were very dry when threshed, injury was more likely to result.

In general, the dry-shell field beans, as a group, seem to be less subject to baldhead than the snap beans, although the comparison has not been carried far enough to justify the drawing of positive conclusions. Out of 13 varieties of field beans investigated, only 3—the Red Kidney, Bayo, and Large White—all grown in California, showed a percentage of baldhead sufficiently large to be of any economic importance. On the other hand, a considerable percentage of baldhead occurred in most varieties of snap beans, but there are a few exceptions, among which may be mentioned the Kentucky Wonder Wax, Tennessee Green Pod, and Lazy Wife.

Several theories have been proposed to explain the general dissimilarity between the dry-shell and snap-bean varieties, but all are untenable or can be negated by several exceptions. The growers of snap beans for seed purposes generally agree that stringless varieties are more difficult to thresh than stringy ones and that the difficulty of threshing is increased if the vines are cut before the pods are entirely ripe. As a consequence the seeds must be released from the pods by speeding up the cylinders or by more nearly closing the concave, both of which may cause some splitting and cracking of the seeds. In threshing the stringless varieties the pods break crosswise at each side of the seed, leaving the bean incased in a segment of the pod. The stringy varieties, among which are the dry-shell field beans, have a large amount of xylem paralleling the dorsal and ventral sutures along which the pods split instead of breaking into segments when struck by the cylinder, thus releasing the beans without too violent treatment. While there seems to be some evidence that baldhead is more prevalent in the stringless varieties than in the stringy ones, the correlation is far from being perfect, since a high percentage of baldhead is sometimes found in some of the stringy varieties and a low percentage in certain lots of some of the stringless ones.

While the stringiness of the bean may partially explain the occurrence of baldhead, undoubtedly other factors, such as the maturity of the crop when harvested and the degree of curing and desiccation of the vine and of the bean itself when threshed, may play important rôles. Information acquired from reliable sources indicates that beans cut a little too green are difficult to thresh, due to the fact that the pods dry tightly about the beans, especially if weather conditions are unfavorable for curing.

Much of the snap-bean seed is grown in the arid regions of the West, where the annual rainfall alone is insufficient to grow a crop and water is supplied by irrigation several times during the growing season. After the beans have reached a certain stage of maturity, water is withheld and the crop is allowed to ripen. It sometimes happens that the ripening process is started too soon, or that an unexpected period of warm weather occurs, so that the beans are subject to drought conditions and ripen prematurely. Under such conditions the pods shrink and tighten about the seeds, rendering their release during threshing extremely difficult and causing them to break into 1-seeded

segments instead of cracking along the sutures. In the light of the evidence at hand, the indications are that not one but a combination of seasonal factors render the seed of certain varieties subject to injury by the threshing machine.

SUMMARY

Investigations have been made of baldhead in beans, a seedling abnormality in which the plumule is absent.

Germination tests of snap and field beans, together with histological studies of the epicotyledonary region of embryos from hand-threshed and machine-threshed beans, have shown that the epicotyl is fractured just below the plumule by the threshing machine. Baldhead rarely occurs in beans threshed by hand. Baldhead plants may develop buds in the axils of the cotyledons which may result in a few flowers and possibly one or two partially filled pods, but never a full yield.

The percentage of baldhead varies with the different varieties and ranges from 0 to 30 per cent. Among some of the snap-bean varieties 10 to 20 per cent is common. Baldhead occurs only to a very slight extent or not at all in many of the field or dry-shell beans studied.

The average diameter of the epicotyl in the embryonic stage was found to be less in snap beans susceptible to baldhead than in more resistant field beans.

BACTERIAL LEAF SPOT OF SQUASH¹

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INTRODUCTION

Bacterial leaf spot of squash first came to the attention of the writer in 1925 when specimens were sent to E. F. Smith from Ithaca, N. Y., by C. D. Chupp, who wrote concerning the infected plants:

The disease started in the center of a small planting of Hubbard squash and is spreading rather rapidly in all directions. It kills the foliage and seems to be very virulent.

In reply to a request for further information he wrote:

The plants were not killed entirely, but many of the leaves were so badly blighted that the plants looked almost black. Apparently the bacterium must have been carried in the seed, since none of the Hubbard squash were grown in that vicinity, and so far as I know Hubbard squash had never been planted in that garden before.

It was at first suspected that this was a new host for *Bacterium lachrymans*, but the isolation of a yellow bacterium that was proved pathogenic to squash by inoculation disproved this theory. In a brief description of the organism² the name *Bact. cucurbitae* was proposed. A short account also appeared in the Yearbook of the United States Department of Agriculture.³ The present paper is intended to give a more complete description of the pathogene and of its effect on its hosts.

GEOGRAPHICAL DISTRIBUTION

Since its discovery in New York, bacterial leaf spot of squash has been found in Maryland, the District of Columbia, Georgia, and South Carolina. In New York it has been reported on Hubbard squash only, but in Maryland it was found on Hubbard squash and Boston Marrow (*Cucurbita maxima*), pumpkin, and Golden Summer Crookneck squash (*C. pepo condensata*). In the District of Columbia, Georgia, and South Carolina it has been found only on Golden Summer Crookneck squash.

SYMPTOMATOLOGY

Lesions are confined chiefly to the leaves, but occasionally young stems and petioles are attacked. No infections have been found or produced by inoculation on fruits. Leaf spots are conspicuous, especially in the early stages, for the wide, bright-yellow halo that surrounds them.

Infections are first apparent two or three days after spray inoculation. At that time they show on the lower surface as minute water-soaked areas penetrating to the upper surface as indefinite yellow

¹ Received for publication Sept. 10, 1929; issued February, 1930.

² BRYAN, M. K. BACTERIAL LEAF SPOT ON HUBBARD SQUASH. *Science* (n. s.) 63: 165. 1926.

³ BRYAN, M. K. SQUASH OF HUBBARD VARIETY ATTACKED BY NEW LEAF SPOT. U. S. Dept. Agr. Yearbook 1927: 599-600., illus. 1928.

areas. They enlarge rapidly, so that by the fourth or fifth day there is a definite round or angular, thin, brown, translucent center having on the upper surface a wide yellow halo (fig. 1) and on the lower surface either a water-soaked margin or no differentiation beyond the sunken spot. With further enlargement the spots are angular and restricted by the veins. Single isolated spots may reach a diameter of 6 or 7 mm.

Large dead areas are produced by the coalescing of crowded spots, as shown on the lower part of the inoculated leaf. (Pl. 1.) Here each spot is delimited by the veins which separate it from adjoining spots. The whole area is thin, dry, translucent, and mottled light and dark brown. The same patchwork effect is seen in the original material from Ithaca, N. Y. (Pl. 2.) Although papery, thin, and

dry, the spots do not tear and drop out as in angular leaf spot of cucumber.

Large dead areas also form where injury to veins causes the shriveling of the tissues beyond. This is seen in the tip of the leaf shown in Plate 1, A. Spots and aggregates of spots may be distinguished within this dead, opaque area.

On shaded portions of leaves the spots may be dark gray instead of brown; the yellow halo is as conspicuous on such spots as on those exposed to direct sunlight.

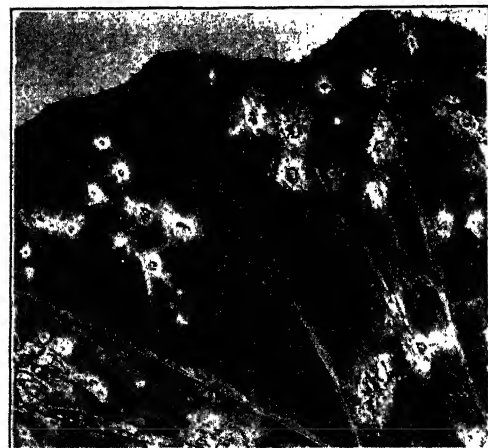
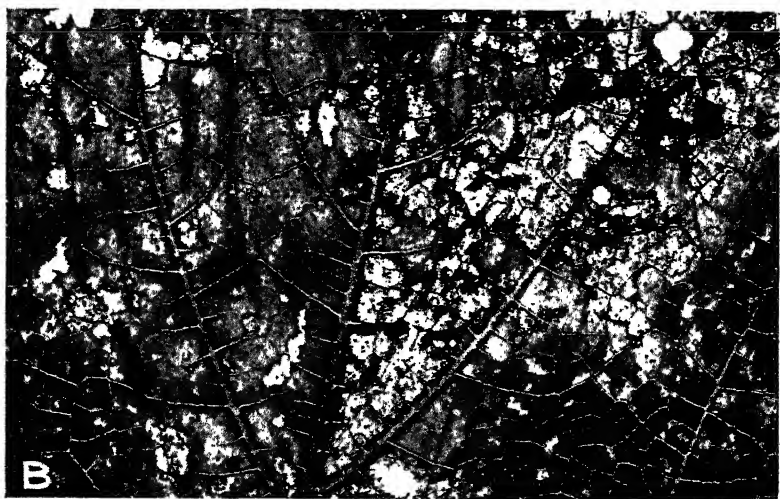
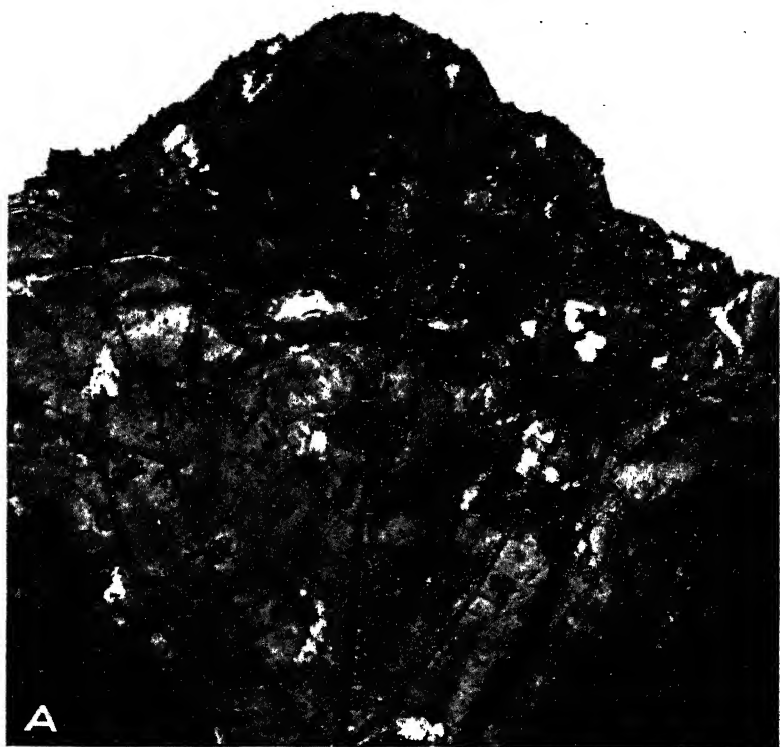


FIGURE 1.—Young infections on a Hubbard squash leaf from a hothouse plant. Collected seven days after spraying with *Bacterium cucurbitae*. $\times 1$

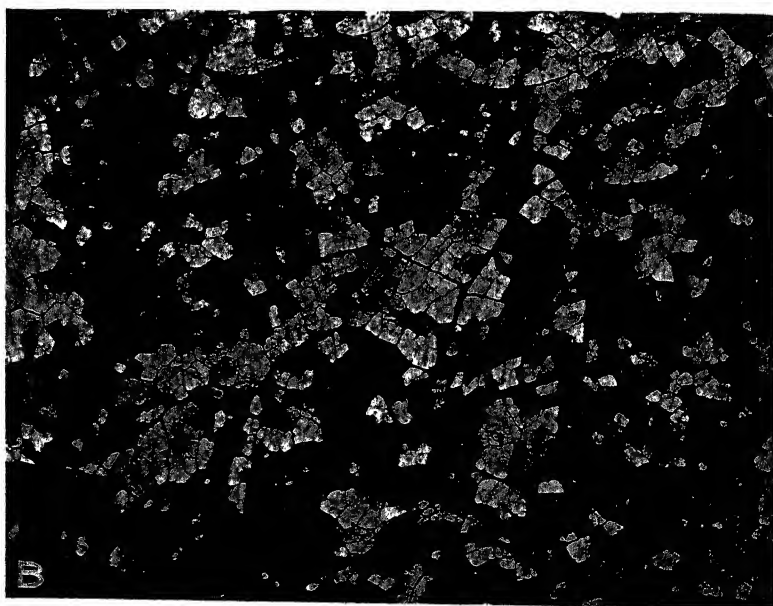
Occasionally young stems of summer squash are attacked (fig. 2) and cracking results. Very young plants may be killed by injury to the growing point when thus infected.

PATHOLOGICAL HISTOLOGY

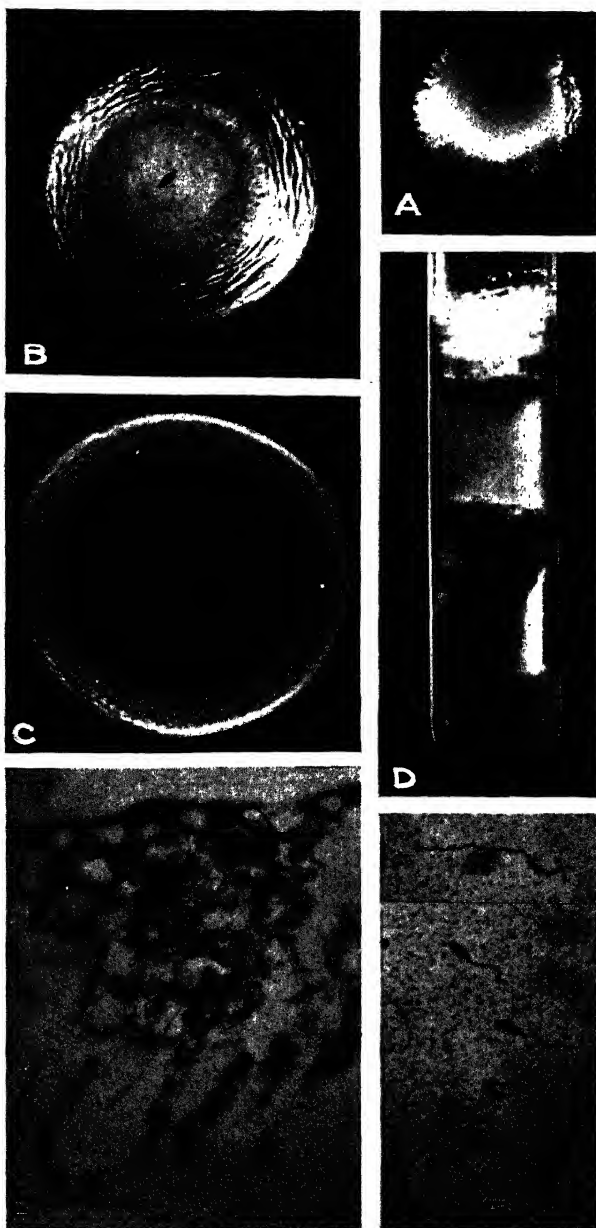
The bacteria enter the leaf by way of the stomata, which are very numerous on both leaf surfaces, especially on the lower one. Bacteria may be demonstrated in the substomatal chamber and penetrating half the thickness of the leaf on the second day (pl. 3, E), after inoculation by spraying. At that time the only outward indication of infection is a slightly water-soaked spot on the lower surface. By the fourth day bacteria have spread in all directions, filling the intercellular spaces of the loose parenchyma in the lower part of the leaf and wedging between the palisade cells to the upper epidermis. In stained sections cut at this stage no collapse has taken place. The cells of the infected area are turgid, but the cell walls in the invaded area take a deeper stain than those in the adjoining tissues. Very shortly after this stage the invaded tissues collapse. This collapse is much more evident on the lower surface than on the upper.



Secondary infections on a Hubbard squash leaf from a plant inoculated with *Bacterium cucurbitae*:
A, Photographed by reflected light; B, by transmitted light. $\times \frac{3}{8}$



Dry leaf, showing natural infection by *Bacterium cucurbitae* on original Hubbard squash leaf from Ithaca, N. Y.: A, Photographed by reflected light; B, by transmitted light. $\times \frac{3}{4}$



A-C.—Agar-plate colonies of *Bacterium cucurbitae* taken by oblique transmitted light: A, 5 days old, $\times 5$; B, 7 days old, $\times 5$; C, 9 days old, $\times 7$.
 D.—Milk culture, 4 weeks old
 E.—Section of Hubbard squash leaf showing stomatal infection two days after inoculation by spraying with *Bacterium cucurbitae*. $\times 400$
 F.—*Bacterium cucurbitae*, showing flagella stained by Casares-Gil's stain. Note the extremely long flagellum on the uppermost rod. These occur frequently on this organism. $\times 940$

Bacterial leaf spot should not be confused with the virus disease described as ring spot by Wingard.⁴ In early stages of systemic ring spot on squash where the water-soaked or necrotic center is of pin-point size with a yellow halo, it might be difficult to distinguish the lesions from those of bacterial spot. Later stages could not be confused. On pumpkin, however, the spotting as shown in Wingard's⁵ Figure 20 is very suggestive of bacterial spot, except that in the latter disease the spots do not follow the veins.

INOCULATIONS

Stomatal infections are readily produced on Hubbard squash by spraying the plants in inoculation cages with a suspension of *Bacterium cucurbitae*. From two to three days elapse between the time of inoculation and the first appearance of lesions. The youngest completely or partially expanded leaves are most susceptible.

Good infections have been obtained in the manner just described on all the varieties of the genus *Cucurbita* on which the disease occurred in the field and also on Italian summer squash, Early White Bush Scalloped squash, Large Field pumpkin, and Sugar Pie pumpkin. Several varieties of *Cucurbita moschata* (Striped Cutshaw, Winter Crookneck, and Large Cheese pumpkin) have been successfully inoculated. *Cucumis* species, such as cucumber (*C. sativus*) and muskmelon (*C. melo*), appear immune. Very minute infections were once produced on cucumber, but they did not develop beyond pin-point size. Good infections have been obtained on watermelon (*Citrullus vulgaris*). (Fig. 3.)

Reisolations were made from spots on each of the successfully inoculated varieties, and the characteristic colonies thus obtained proved infectious on Hubbard squash.



FIGURE 2.—Stem of young Golden Crookneck summer squash infected with *Bacterium cucurbitae*. $\times 1$

⁴ WINGARD, S. A. HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS. Jour. Agr. Research 37: 127-153, illus. 1928.

⁵ WINGARD, S. A. Op. cit.

Seed inoculated with *Bacterium cucurbitae*, dried and planted, have yielded as high as 15 per cent infection, the disease sometimes showing first on the cotyledons and at other times on the first leaves.

Inoculations with *Bacterium cucurbitae* both by spraying on and pricking in the inoculum, on cotton, cabbage, string bean, Lima bean, and poppy, all of which are hosts of other yellow plant patho-

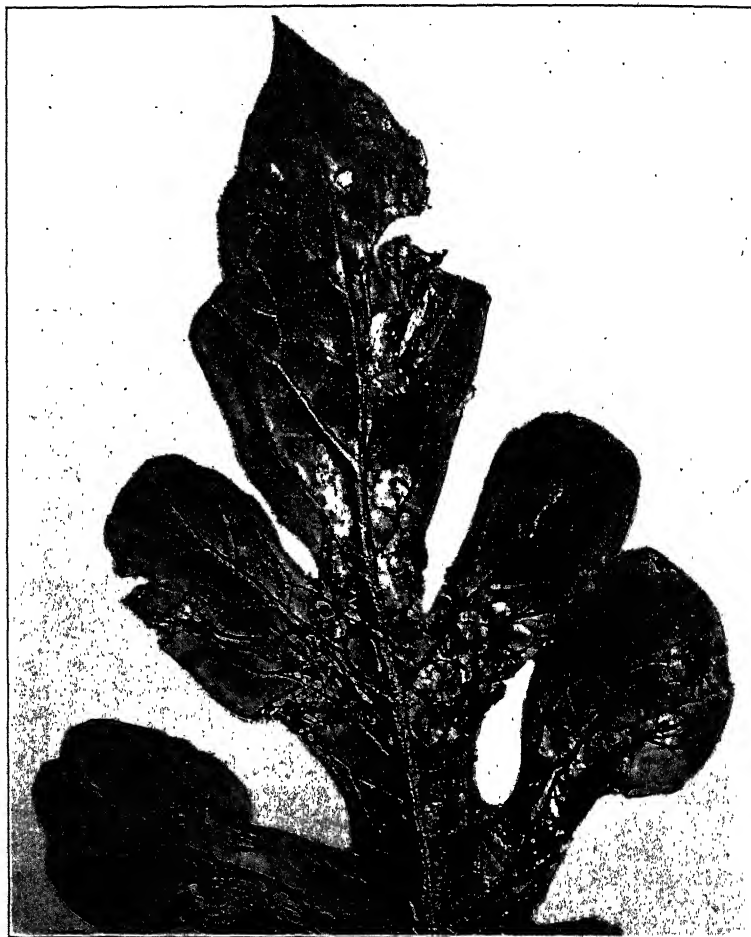


FIGURE 3.—Watermelon leaf five days after spraying with *Bacterium cucurbitae*. $\times 1\frac{1}{4}$

genes, have been entirely negative. Good infections resulted on Hubbard squash used as checks.

METHOD OF DISSEMINATION

The organism is apparently carried on the seed, but it does not appear to infect the soil. Although weather conditions favored infection, perfectly healthy Hubbard squash vines were grown on a plot in the District of Columbia where the disease had been very

severe the previous year. Further observations are needed to complete the evidence on these points. Spread in the field is facilitated by weather conditions, chiefly by rain. Potted plants of Hubbard squash, with several infected leaves each, set out in the ground early in July, developed vigorous healthy vines with no new infections during a long period of dry weather, but during a 10-day rain in August following this drought heavy infection spread rapidly through the patch and continued to spread with succeeding rains. Newly infected leaves appeared adjacent to old infections, and although squash bugs, cucumber beetles, and aphids were abundant on the vines, no infections were found where they fed on the leaves, at a distance from infected leaves. It would seem, therefore, that these insects do not spread the disease.

THE PATHOGENE

MORPHOLOGY

Bacterium cucurbitae is a short, polar-flagellate rod, 0.5μ to 1.3μ by 0.45μ to 0.6μ , usually with only one flagellum, which is frequently of unusual length. (Pl. 3, F.) It occurs singly, in pairs, or in short chains. Capsules are formed, but no spores.

REACTION TO STAINS

This organism stains readily with the usual bacterial stains, is definitely Gram negative, and is not acid fast.

CULTURAL CHARACTERS*

BEEF-AGAR PLATES.—Colonies on thinly sown plates are visible on the third day. They are less than 1 mm. in diameter, round, pale yellow, opalescent, and finely crosshatched or without markings. By the fifth day they are 3 or 4 mm. in diameter, showing by transmitted light an opaque center and a thinner margin, which may be either crosshatched or show concentric lines. (Pl. 3, A.) Some colonies are slightly umbonate. Later they may reach a diameter of 6 or 7 mm. and become decidedly umbonate. The concentric lines or crosshatching persists, and the opalescence remains very striking. (Pl. 3, C.) Sometimes fine radiating lines appear in the margin (pl. 3, A) or as a ring between the dense center and the thin margin. (Pl. 3, B.) The color, at first wax yellow, becomes mustard yellow.⁷ The consistency is at first viscid and later butyrous. On plates 2 weeks old secondary growths of deeper color have formed in the colonies. Buried colonies are lenticular; bottom colonies thin, white, and granular with one or two concentric rings.

BEEF-AGAR SLANTS.—Growth is moderate, 2 to 6 mm. wide, mustard yellow, opalescent, and smooth with undulating margins. The consistency is viscid at first, but it becomes butyrous. Usually no crystals are formed, but occasionally a few small crystals appear just below the surface of the slant.

BEEF-EXTRACT AGAR.—Growth on beef-extract agar is deeper yellow, more restricted with less noticeable markings and less conspicuous opalescence than on beef-infusion agar.

BEEF BROTH.—Clouding is prompt but moderate. A rim of coarse pseudozoo-gloesae is formed, and in undisturbed cultures also a partial or complete pellicle of the same composition. Cultures 3 weeks old or more are clear or almost so and have a heavy yellow precipitate, which rises in a viscid swirl on shaking. Numerous small crystals lie in the precipitate.

POTATO-DEXTROSE AGAR.—On this medium growth is abundant, soon covering the entire surface with a heavy, butyrous, pale-yellow layer of growth.

* Unless otherwise stated, all beef media were made with beef infusion and had a pH of 6.3 to 7.2.

⁷ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912

POTATO CYLINDERS.—On steamed-potato cylinders growth is very heavy, butyrous, and bright yellow, covering the potato and filling the water with a dense yellow slime, eventually almost or completely submerging the potato, which shrinks with the destruction of the starch. Old cultures take on a more or less deep-brown tinge.

SYNTHETIC MEDIA.—In Cohn's solution growth is very feeble or absent. Very moderate growth occurs in Ushinsky's solution. In Fermi's solution cultures cloud moderately. In none of these media is there any rim, pellicle, precipitate, or fluorescence.

PHYSIOLOGY

LIQUEFACTION OF GELATIN.—In gelatin stabs at 20° C. liquefaction begins on the second day, is saucer shaped, becoming stratiform, and is only half complete in one month. At this stage there are an abundant yellow precipitate and a yellow rim, but the liquefied gelatin is practically clear. Liquefaction is complete in six to eight weeks.

LIQUEFACTION OF BLOOD SERUM.—Clearing of the serum begins on the fourth day, followed on the fifth by liquefaction, which extends throughout the slanted portion and slightly below; but neither process reaches the bottom of the tube.

FERMENTATION OF SUGARS.—The ability of the organism to ferment various carbohydrates was tested on peptone-free synthetic agar⁸ with brom-cresol purple as an indicator. One per cent of each of the following was used: Sucrose, dextrose, lactose, maltose, galactose, levulose, glycerin, and mannitol. Acid was formed from all except mannitol, beginning on the seventh day in sucrose, dextrose, galactose, and maltose, and in nine days in lactose, glycerin, and levulose. The cultures never became acid throughout, the lower portions remaining pale purple.

When the same carbohydrates were tested in beef-extract agar with brom-cresol purple as indicator no acid was evident at any time; in the same beef-extract agar with the same carbohydrates and with phenol red as indicator, an alkaline reaction began within 24 hours and within 10 days had spread throughout the agar in all cultures.

No gas was formed in fermentation tubes from any of these carbohydrates, nor was there any growth in the closed end.

HYDROLYSIS OF STARCH.—The starch of potato cylinders is almost completely destroyed and the shrunken cylinder submerged in the abundant yellow growth. On starch-agar plates a clear area 2 to 2.3 cm. wide around the streak of bacterial growth is produced within seven days.

RELATION TO FREE OXYGEN.—The organism is aerobic; it does not grow in the closed end of fermentation tubes and makes no growth in the lower half of agar stabs or in the lower part of shake-agar cultures.

NITRATE REDUCTION.—Nitrates are not reduced.

REACTIONS IN MILK.—No acid is formed in milk; peptonization sets in on the fifth or sixth day and is complete in eight days. Separation begins at about this time. At the end of three weeks the cultures present the appearance shown in Plate 3, D, with a heavy yellow rim above a layer of clear whey; below this is a heavy yellow precipitate on top of the soft curd, which has drawn away from the sides of the tube. Occasionally a few tyrosine crystals are formed. Reduction of methylene blue begins in three days and is complete in seven days. Reduction of litmus begins by the tenth day and is complete by the fourteenth day.

PRODUCTION OF AMMONIA.—A moderate amount of ammonia is produced in beef media.

PRODUCTION OF HYDROGEN SULPHIDE.—Hydrogen sulphide is produced in beef broth. Tests with strips of lead-acetate paper inserted in beef-broth cultures became a deep brown by the sixth day.

PRODUCTION OF INDOL.—No indol is produced.

TOLERATION OF SODIUM CHLORIDE.—Clouding is prompt and heavy in beef broth containing 1 and 2 per cent NaCl. It is retarded slightly in the presence of 3, 4, and 5 per cent. Growth in 5 per cent remains very moderate and contains many chains.

OPTIMUM pH FOR GROWTH.—The organism makes its best growth in pH 6.5 to 7.0. The limits for growth are pH 5.8 and pH 9.0.

TEMPERATURE RELATIONS.—The best growth takes place at 25° to 30° C., no growth occurs above 35°, and the thermal death point is 49°.

LONGEVITY ON CULTURE MEDIA.—Cultures of this organism are shorterlived on culture media than most pathogenes; even beef-agar stabs, when kept in the

⁸ SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIC. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA . . . 48 p., illus. Geneva, N. Y. [1923.]

ice box, must be transferred every three or four months, as after six months without transfer most strains are dead.

TECHNICAL DESCRIPTION

Bacterium cucurbitae is a short rod $0.5\ \mu$ to $1.3\ \mu$ by $0.45\ \mu$ to $0.6\ \mu$, with one polar flagellum, occurring singly, in pairs, or in short chains. It forms no spores, is not acid fast, and is Gram negative. On beef agar it forms round, yellow, opalescent colonies with internal striae. Its diastasic action is strong. It liquefies gelatin and blood serum slowly. Acid without gas is formed from sucrose, dextrose, lactose, maltose, galactose, levulose, and glycerin, but not from mannit. It does not form acid in milk, but reduces litmus and methylene blue in this medium. It does not reduce nitrates or form indol, but it does produce ammonia and hydrogen sulphide. Its optimum pH or growth is 6.5 to 7.0. Its optimum temperature is 25° to 30° C., its thermal death point being 49° .

SUMMARY

This paper describes a bacterial leaf spot which attacks summer and winter squashes and pumpkins. The disease can be transmitted to watermelon by inoculation, but it has not been found occurring on this plant in the field. It has not been found on cucumbers or muskmelons, and inoculations have failed to produce it on them.

The leaf spots are brown surrounded by a yellow halo, and they do not tear or drop out as do those of angular leaf spot of cucumbers. They often coalesce to form large dead areas.

The disease appears to be seed borne. The evidence at hand indicates that it does not live over winter in the field and is not transmitted by insects. It spreads rapidly in rainy weather.

A description is given of *Bacterium cucurbitae*, the causal organism.

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FRUIT-BUD DEVELOPMENT IN STRAWBERRY VARIETIES AND SPECIES¹

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INTRODUCTION

Investigations in fruit-bud formation in the strawberry have hitherto been confined to a few well-known varieties. The results obtained from such investigations have greatly aided both the breeder of strawberries and the investigator studying cultural practices. However, in order better to understand the relationship of fruit-bud formation to cultural practices when applied to different varieties, it has been found necessary to determine how fruit-bud initiation occurs and development proceeds in a wide range of variety types and species.

This report is the result of a study of the course of fruit-bud development in representative varieties and in several species, made at the United States Plant Field Station, Glenndale, Md., about 15 miles from Washington, D. C.

HISTORY

The time of fruit-bud formation in the strawberry was first studied in Wisconsin by Goff,² who found that in 1899 in the Clyde variety it occurred in late September. Morrow³ in Iowa in 1906 found traces of fruit-bud differentiation in the Dunlap and Warfield varieties by September 18. Studies by Hill and Davis⁴ at Ottawa, Canada, indicate that fruit-bud differentiation occurs about September 19. Ruef and Richey⁵ in Iowa found fruit-bud formation in the Dunlap taking place by September 7. Darrow⁶ concluded that in certain parts of California fruit-bud formation must occur throughout the summer and probably during the winter months, as the fruit continues to mature throughout the year. From a survey made in 1923 of several varieties of strawberries at different experiment stations from Massachusetts to Minnesota, Darrow⁷ concluded that fruit-bud differentiation takes place during the latter part of September, occurring slightly in advance in the early varieties. He stated that in all sections fruit buds were visible to the unaided eye by the middle of October.

¹ Received for publication June 12, 1929; issued March, 1930.

² GOFF, E. S. INVESTIGATIONS OF FLOWER BUDS. Wis. Agr. Expt. Sta. Ann. Rpt. 17: 266-285, illus. 1900.

³ MORROW, H. E. A STUDY OF THE FORMATION OF FLOWER BUDS WITH SPECIAL REFERENCE TO THE STRAWBERRY. 1907. [Unpublished thesis. Iowa State Col. Agr. and Mechanic Arts, Ames.]

⁴ HILL, H., and DAVIS, M. B. STUDIES IN STRAWBERRY BUD DIFFERENTIATION. Canada Dept. Agr. Bul. (n. s.) 110, 15 p., illus. 1929.

⁵ RUEF, J. U., and RICHEY, H. W. A STUDY OF FLOWER BUD FORMATION IN THE DUNLAP STRAWBERRY. Amer. Soc. Hort. Sci. Proc. 22: 252-260. 1925.

⁶ DARROW, G. M. ESSENTIALS IN STRAWBERRY CULTURE. PART III. FRUIT BUD FORMATION. Amer. Fruit Grower Mag. 46 (6): 7, 18-19, illus. 1926.

⁷ See footnote 6.

MATERIALS AND METHODS

The studies herein reported were conducted during the late summer and autumn months of 1926, 1927, and 1928. The investigations made in 1926 and 1927 were more or less preliminary to the more complete studies made in 1928. Only representative varieties and species of the various types of strawberries were selected. Early varieties such as the Campbell and Excelsior, late varieties such as the Chesapeake, varieties grown in the South such as Klondike and Missionary, northern varieties such as Dunlap and Portia, and Ananas de Guemene, a European variety, were studied.

Selection No. 27 from Highlands, N. C., was chosen as representative of *Fragaria virginiana* Duchesne, the wild strawberry of the eastern part of the United States. This selection is late in maturing, and it is possible that earlier forms of this species might have been found. *F. chiloensis* (L.) Duchesne, which is native to the Pacific coast, is probably always late in maturing. The plants used were the progeny of plants from the beaches of Oregon. The one form of *F. nilgerrensis* Schlechtend, a species native to northern India, which was available, was late. *F. americana* (Porter) Britton is an American representative of *F. vesca* L., the wood strawberry of Europe, and matures its fruit late.

The varieties of strawberries studied in 1927 and 1928, which include most of those previously reported by other investigators, are listed below. Howard 17 was the only variety studied in 1926.

1927	U. S. D. A. No. 25. U. S. D. A. No. 854. Vantage. White sugar.
Ananas de Guemene. Black Hautbois (<i>Fragaria moschata</i>). Belt. Campbell. Chesapeake. Dunlap. Erige du Poitou (<i>F. vesca</i>). Ettersburg 80. Ettersburg 121. Excelsior. <i>F. americana</i> . <i>F. chiloensis</i> . <i>F. nilgerrensis</i> . <i>F. virginiana</i> No. 27. Heflin. Howard 17. Joe. John Ruskin. Kalicene. Little Scarlet (<i>F. virginiana</i>). Lupton. Missionary. Monstrueuse Hautbois (<i>F. moschata</i>). Portia. Sample.	1928
	Ananas de Guemene. Aroma. Campbell. Chesapeake. Dunlap. Erige du Poitou (<i>F. vesca</i>). Excelsior. <i>F. americana</i> . <i>F. chiloensis</i> . <i>F. nilgerrensis</i> . <i>F. virginiana</i> No. 27. Heflin. Howard 17. Kalicene. Klondike. Missionary. Monstrueuse Hautbois (<i>F. moschata</i>). Portia. Sample. U. S. D. A. No. 25.

In 1927 material was collected every 15 days, beginning September 1 and ending October 15. In 1928 collections were made almost continuously from late August until early in November.

Studies in 1926 and 1927 followed the usual method of killing, fixing and embedding in paraffin, sectioning, and staining. Both Haidenhain's iron-alum and Delafield's haematoxylin were used, and good results were obtained by both stains. However, it was very

evident that the above methods were not adapted to the study of large numbers of buds. The results of the work in 1927 indicated that fruit-bud development, at least in the strawberry, is too variable to permit definite conclusions as to the time and rate of development to be drawn from an examination of a limited quantity of material.

During the season of 1927 runner plants of nearly the same age which had taken root early in August were used for these studies. The results obtained in 1927, however, were somewhat variable. It was considered that the variability might have been due in part to slight differences in age and in part to growing conditions not favorable to root formation by young plants. Therefore, in order to obtain more uniformity, a special planting was made in April, 1928, on a clay loam soil as uniform as it was possible to obtain, and only the mother plants from this plot were used for the studies herein reported.

It was found that, with the use of a very sharp razor, free-hand sections of as many crowns or growing points as were necessary could be cut in a short time and thin enough to give a clear idea of the range and stage of development at any particular time. As soon as cut, these sections were put in 95 per cent alcohol for 10 to 15 minutes, after which they were passed through 50 per cent alcohol, washed in water, and stained in Delafield's haematoxylin for 3 to 5 minutes. After staining, the sections were washed in water and passed through a series of gradually ascending strengths of alcohol to absolute alcohol, where they were allowed to remain about 5 minutes, then removed to xylol, and finally mounted in Canada balsam on slides.

When development had reached a stage where it was no longer necessary to use a microscope to see the fruit buds, several crowns of each variety were cut lengthwise, and the most representative were selected for photographing. It was found that if the cut tissue was allowed to oxidize until it had become a light brown the structures showed best. Photographs three times the actual size were then obtained. Camera-lucida drawings of the mounted sections were made for comparison. It was possible to classify the early stages by the use of the drawings and the later stages by the photographs.

PROCESS OF FRUIT-BUD FORMATION

Goff² regarded the broadening of the crown or growing point accompanied by the assumption of an irregular outline as an indication of an early stage of flower development in the strawberry. For these studies definite fruit-bud differentiation was considered to have taken place in the strawberry when the growing point had become broad and somewhat flattened on top. At this stage elongation begins immediately, and when the flower stalk starts to lengthen new growing points appear at the base, which develop into secondary flowers or other flower stalks. Further elongation results in tertiary and quaternary flowers differentiating on the flower stalk just behind the secondary and primary flowers.

Ruef and Richey³ state that the degree of development of the flower parts varies according to the respective flower buds on the fruit stalk. They also give the order of development as sepals, petals, stamens,

² See footnote 2.

³ See footnote 5.

and pistils. Their findings are corroborated in the present study, for soon after the elongation of the flower stalk begins the rudiments of the sepals appear, followed quickly by the petals and stamens. Some time elapses after rudimentary sepals, petals, and stamens have differentiated before the pistils appear on the receptacle. Pistil development begins on the edges of the receptacle and advances toward the center. It is following the appearance of these pistils on the primary flower that rapid elongation of the flower stalk begins.

INDICATIONS OF FRUIT-BUD FORMATION

It was found that the broadening of the growing point, which is considered the first definite indication of fruit-bud differentiation, is preceded (1) by the thickening of the crown, so that the last differentiated rudimentary leaves appear almost on the same level as the vegetative growing point, and (2) by the growing point itself beginning to be dome shaped. These indications were most noticeable in Howard 17, Campbell, and Kalicene in the latter part of August. Perhaps the crowns of other varieties and species thicken in some degree during the fall months under Maryland conditions.

Such indications as have been described occurred over a period of a few days in some varieties to as much as one month in others before definite fruit-bud differentiation could be seen. Some idea of the conditions found during the 1928 season may be obtained from Table 1. The periods given in the table are not assumed to be indicative of the development of all buds, for during the same period many crowns were found with typical vegetative growing points.

TABLE 1.—*Period covered by indications of fruit-bud differentiation and the approximate date of definite fruit-bud formation in 14 varieties and 4 species of strawberries in 1928*

Variety or species	Period covered by indications of fruit-bud differentiation	Date of definite differentiation in the majority of the crowns	Variety or species	Period covered by indications of fruit-bud differentiation	Date of definite differentiation in the majority of the crowns
Ananas de Guemene.....	Sept. 24-Oct. 5	Oct. 6	Fr. virginiana No. 27.....	Aug. 28-Oct. 1	Oct. 4
Aroma.....	Sept. 6-Oct. 3	Oct. 3	Heflin.....	Aug. 28-Oct. 1	Oct. 1
Campbell.....	Aug. 25-Sept. 15	Sept. 18	Howard 17.....	Aug. 25-Sept. 14	Sept. 14
Chesapeake.....	Sept. 7-Oct. 4	Oct. 4	Kalicene.....	Sept. 6-Sept. 27	Sept. 25
Dunlap.....	Aug. 30-Sept. 27	Sept. 27	Klondike.....	Sept. 7-Sept. 26	Oct. 3
Excelsior.....	Sept. 19-Oct. 5	Oct. 5	Missionary.....	Sept. 18-Sept. 27	Sept. 27
Fragaria americana.....	Sept. 19-Oct. 5	Oct. 5	U. S. D. A. No. 25.....	Aug. 27-Sept. 22	Sept. 20
F. chiloensis.....	Oct. 10-Nov. 20	Nov. 6	Portia.....	Aug. 28-Sept. 27	Sept. 27
F. nilgerrensis.....	Oct. 10-Nov. 20	Nov. 20	Sample.....	Sept. 18-Sept. 27	Sept. 23

It can not be concluded that all growing points take 15 to 30 days to pass from the stage of the first noticeable broadening of the growing point until definite elongation of the flower stalk is noticeable. Doubtless some of the early broadening crowns actually differentiated before the date set when definite differentiation was most general. Figure 1 shows the growing point of the Kalicene variety already beginning to elongate on September 1, 1927. Howard 17 and

Campbell also showed the beginning of definite fruit-bud formation on this date. It is believed that some unusual conditions existed in 1927 which caused a few crowns to differentiate fruit buds earlier than is usual for these varieties. In 1928 Howard 17 and Campbell showed indications of fruit-bud differentiation by September 1 and Kalicene by September 6, but no definite fruit-bud development was found in the above three varieties until September 14, or later.

DEFINITE FRUIT-BUD FORMATION

There is little doubt that fruit-bud differentiation has already taken place when the growing point becomes as broad and flattened on the top as Figure 1 shows. At this stage elongation begins immediately. Table 1 gives the approximate dates of definite fruit-bud differentiation in most of the 1-year-old mother plants of each variety studied in 1928. Table 2 gives the dates when definite differentiation was found in 1927.

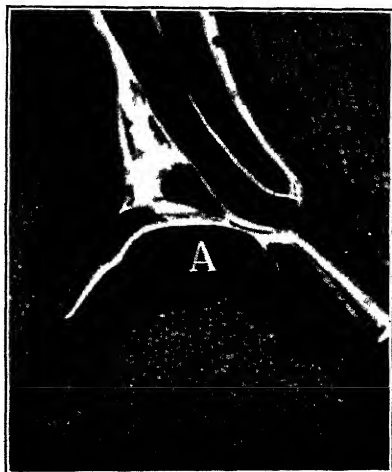


FIGURE 1.—First evidence of definite fruit-bud differentiation in the Kalicene strawberry: Growing point (A) just beginning to elongate, September 1, 1927. $\times 80$

Definite fruit-bud formation was found during the latter half of September and early in October in all varieties and species studied with but one exception. These results are in close agreement with those of Darrow,¹⁰ who concluded that fruit-bud differentiation took place during the latter part of September in sections of the northern United States.

TABLE 2.—Approximate dates when definite fruit-bud formation was found in most of the crowns of 10 varieties of strawberries in 1927

Variety	Definite differentiation indicated by elongation of growing point	Secondary and tertiary flower stalks appearing	Variety	Definite differentiation indicated by elongation of growing point	Secondary and tertiary flower stalks appearing
Campbell.....	Sept. 1	Oct. 1	John Ruskin.....	Oct. 1	Oct. 15
Ettersburg 80.....	Oct. 15	Nov. 3	Kalicene.....	Sept. 1	Oct. 1
Ettersburg 121.....	do	-----	Missionary.....	Oct. 1	Oct. 17
Heflin.....	Sept. 15	-----	U. S. D. A. No 25.....	Sept. 15	Oct. 1
Howard 17.....	Sept. 1	Sept. 15	Sample.....	Oct. 1	Oct. 17

Fruit-bud differentiation was found to cover a considerable period in some varieties, Kalicene being notable in this respect. In this variety differentiation was spread over a period of one month, crowns

¹⁰ DARROW, G. M. [Letters reporting to the cooperating experiment stations.] 1923.

of some plants showing the first stages one month earlier than others. *Fragaria chiloensis* was also found to be somewhat variable, but in general the varieties differentiating first were more variable than those differentiating later.

The species were all found to be late in differentiating (Table 3), particularly *Fragaria nilgerrensis*, native to northern India. This species did not show any definite differentiation until November 27, when development indicated that probably it took place about November 20. Although fruit-bud differentiation in different plants of all the varieties studied was spread over a period of about three weeks, it is possible to classify them roughly according to the time when the initiation process is most general. Such a classification is shown in Table 4. The more limited studies in 1926 and 1927 seem to verify this grouping.

TABLE 3.—Classification of varieties and species of strawberries according to the time of the initiation of the process of fruit-bud formation in 1928 ^a

Early	Midseason	Late	Very late
Campbell. Howard 17. Kalicene. Sample. U. S. D. A. No. 25.	Dunlap. Excelsior. Heflin. Missionary. Portia.	Aroma. Ananas de Guemene. Chesapeake. Klondike. Fragaria chiloensis. F. americana. F. virginiana No. 27.	<i>Fragaria nilgerrensis</i> .

^a Additional studies made in the fall of 1929 on these varieties indicate that this classification is the same for 1929, except for Excelsior, which was in the early class, and Sample, which was in the midseason or late class. Studies in 1929 indicate the following classification of other varieties:

Early	Midseason	Late
U. S. D. A. No. 652. U. S. D. A. No. 655. U. S. D. A. No. 542. U. S. D. A. No. 668. Gene.	Blakemore. Oregon. U. S. D. A. No. 672. U. S. D. A. No. 632.	Bedarena. Sybil. F. vesca type from Grangeville, Idaho. Pearl. Judith. Joe. Ettersburg 121. U. S. D. A. No. 845 U. S. D. A. No. 44.

DEVELOPMENT OF THE FRUIT BUD

It is during the development of fruit buds that the most significant differences between varieties appear. In order to show this trend, a classification is given of the different stages in the process that take place during the autumn. Table 4 gives the dates at which the different varieties and species reached the various stages. Figure 2 shows graphically the process of development in a few representative varieties and species as illustrated in Figures 3 to 9.

Table 5 gives the interval in days between the different stages in the process of development for the varieties and species studied. This classification was based entirely upon the development of the primary flower. It was found from a limited study that the development in the primary, secondary, and tertiary flowers in general is proportional, and is in agreement with the results of Ruef and Richey ¹¹ with the Dunlap strawberry.

¹¹ See footnote 5.

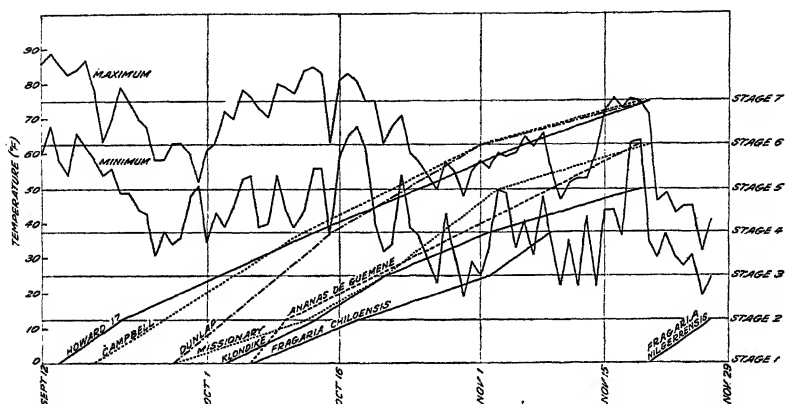


FIGURE 2.—Graphic presentation of the trend of development in the fall of 1928 in certain strawberry varieties representative of those studied



FIGURE 3.—Definite fruit-bud differentiation of the Sample strawberry, representing stage 1 in Figure 2, October 1, 1927. $\times 80$



FIGURE 4.—Rudiments of first, secondary, and tertiary flowers appearing on the Sample strawberry, representing stage 2 in Figure 2, September 26, 1928. $\times 80$



FIGURE 5.—Rudimentary flower parts, except pistils, on the Missionary strawberry, representing stage 8 in Figure 2, October 15, 1927. $\times 80$



FIGURE 6.—First rudimentary pistils appearing on the primary flower of the Campbell strawberry, representing stage 4 in Figure 2, October 1, 1927. $\times 80$



FIGURE 7.—Rapid elongation of the primary flower stalk of the Howard 17 strawberry just beginning, representing stage 5 in Figure 2, November 1, 1928. $\times 3\frac{1}{2}$



FIGURE 8.—Elongated flower stalk of the Missionary strawberry, buds formed but not ready to open, representing stage 6 in Figure 2, November 20, 1923. $\times 3\frac{3}{4}$



FIGURE 9.—Primary flowers of the Excelsior strawberry ready to open, representing stage 7 in Figure 2, November 20, 1923. $\times 3\frac{3}{4}$

TABLE 4.—Approximate dates upon which the 14 varieties and 4 species of strawberries were found to be in the various stages of development in 1928

Variety or species	Primary, secondary, and tertiary flowers appearing	Rudimentary flower parts present, except pistils	First pistils appearing on primary flowers	Rapid elongation of primary flower stalk begun	Buds formed but not open	Primary flower buds ready to open or already in bloom
Aroma.....	Oct. 10	Oct. 18		Nov. 5	Nov. 20	
Ananas de Guemene.....	do.....	Oct. 20	Nov. 2	Nov. 9	Nov. 19	
Campbell.....			Oct. 12		Nov. 1	Nov. 19
Chesapeake.....	Oct. 10	Oct. 20		Nov. 20		
Dunlap.....			Oct. 15		Nov. 1	Nov. 19
Excelsior.....	Oct. 4	Oct. 20				Nov. 15
Fragaria americana.....	Oct. 10	Oct. 22	Nov. 2		Nov. 19	
F. chiloensis.....	Oct. 13	Nov. 2	Nov. 9			
F. nilgerrensis.....	Nov. 27					
F. virginiana.....	Oct. 12	Oct. 20		Nov. 19		
Heflin.....	Oct. 10	Oct. 15		Nov. 1	Nov. 19	
Howard 17.....	Sept. 21		Oct. 13	do.....	Nov. 6	Nov. 20
Kalicene.....	Sept. 30	Oct. 13			Nov. 1	Do.
Klondike.....	Oct. 13	Oct. 20	Nov. 19			
Missionary.....	Oct. 12	do.....	Nov. 1	Nov. 1	Nov. 20	
U. S. D. A. No. 25.....	Sept. 25	Oct. 10	Oct. 15		Nov. 1	Nov. 20
Portia.....	Oct. 13			Nov. 6	Nov. 18	
Sample.....		Oct. 20			Nov. 20	

In general, the degree of development reached at the end of the growing season of 1928 was in more or less correlation with the time of fruit-bud differentiation. But from Figure 2 it appears that under the conditions of these experiments different varieties follow different courses of development. Missionary and Dunlap, for example, begin fruit-bud differentiation about the same time. Development immediately following differentiation in Missionary is somewhat slow compared with that in Dunlap; later, its development becomes very rapid for a time and finally slows up. In contrast, the fruit bud of the Dunlap makes a rapid development at first which gradually becomes relatively slower toward the end of the season. Howard 17, although beginning early, never at any time develops very rapidly. However, because of its early start, it is one of the most fully developed varieties throughout the season.

TABLE 5.—Approximate number of days from date of definite fruit-bud differentiation of strawberries to the various later stages of development in 1928

Variety or species	Primary, secondary, and tertiary flowers appearing	Rudimentary flower parts present, except pistils	First pistils appearing on primary flowers	Rapid elongation of primary flower stalk begun	Buds formed but not open	Primary flower buds ready to open or already in bloom
Aroma.....	7	15	25	33	47	
Ananas de Guemene.....	4	14	26	33	43	
Campbell.....	3	16	24	34	44	62
Chesapeake.....	6	16	32	47		
Dunlap.....	6	12	18	26	35	54
Excelsior.....	6	22	29	35	41	48
Fragaria americana.....	5	17	28	36	45	
F. chiloensis.....	12	27	34			
F. nilgerrensis.....	7					
F. virginiana.....	8	16	31	46		
Heflin.....	9	14	22	30	48	
Howard 17.....	7	18	29	48	53	67
Kalicene.....	5	18	24	31	37	56
Klondike.....	10	17	30	47		
Missionary.....	15	23	29	35	55	
U. S. D. A. No. 25.....	5	20	25	33	42	56
Portia.....	16	24	32	40	52	
Sample.....	14	27	38	48	58	

Of even more significance than the irregularity in the rate of development is the interval of time between the different stages, which for all varieties studied averages about 10 days between successive stages. Some varieties develop consistently faster than this, others slower. Table 6 groups the varieties according to their rate of development. By comparing Table 4 with Table 6, it will be noted that certain varieties differentiate their fruit buds early and continue to make rapid growth throughout the season; such varieties are Campbell, Kalicene, and U. S. D. A. No. 25 (a cross of Van Fleet 3 × Campbell, originated at the United States Plant Field Station, Glenndale, Md.).



FIGURE 10.—Primary flower buds of the Dunlap strawberry just beginning to open: A, November 8, 1927; $\times 1\frac{1}{4}$. B, November 20, 1928; $\times 3\frac{3}{4}$

Howard 17 makes a slow growth and Sample a much slower growth. Dunlap and Excelsior, although differentiating fruit buds later than the sorts above mentioned, develop rapidly and at the end of the season attain the same degree of growth as Campbell.

Ananas de Guemene and *Fragaria americana*, although among the latest to differentiate fruit buds, develop very rapidly in the late fall. By November 8, 1927, there was an occasional open bloom on *F. americana*. Chesapeake, Klondike, and *F. virginiana* differentiate fruit buds late and make a more or less slow growth thereafter.

Figures 10 to 12 show a very striking similarity between the seasons of 1927 and 1928 in the degree of development in the same varieties. The primary flower occasionally comes into blossom in late fall. Secondary flowers almost never blossom, unless, perhaps, when weather conditions are very abnormal.



FIGURE 11.—Flower buds of the U. S. D.A. No. 25 strawberry in various stages of development: A, November 15, 1927; $\times 134$. B, November 20, 1928; $\times 314$



FIGURE 12.—Opening blossoms of Campbell strawberry: A, November 8, 1927. B, November 19, 1928; $\times 134$

TABLE 6.—*Classification of varieties and species of strawberries according to the rate of development of their fruit buds in autumn, season of 1928*

Rapid	Intermediate	Slow
Ananas de Guemene. Campbell. Dunlap. Excelsior. Fragaria americana. Kalfoene. U. S. D. A. No. 25.	Aroma. Heflin. Howard 17. Missionary. Portia.	Chesapeake. Klondike. Sample. Fragaria chiloensis. F. virginiana.

In late November, 1928, open blossoms were found on some plants of many varieties, particularly Campbell, Dunlap, Excelsior, U. S. D. A. No. 25, and Howard 17. Somewhat similar conditions were found in November, 1927. As these flowers are almost always primary ones that are killed during the late fall, the secondary flowers on these plants may not open in the following spring before the primary flowers of some other variety not so well advanced at the end of the fall growing season.

Significant differences between varieties are even more striking when the ripening season is considered. In Table 7 the varieties and species are classified according to their ripening season. It will be noted that Campbell and U. S. D. A. No. 25, which were among the first to differentiate, are also among the first to ripen. Howard 17, the first variety of all to begin differentiation, is one of the first to ripen, but it does not develop as rapidly in the autumn as some other varieties, and although early blooming it is not among the very first to bloom in the spring. Dunlap, which differentiates fruit buds much later than Howard 17 but which develops rapidly and blooms early in the spring, does not ripen as early as Howard 17 under Maryland conditions, although it is early in the North-Central States, where it originated.

TABLE 7.—*Classification of varieties and species of strawberries according to their ripening season*

Early	Midseason	Late	Very late
Campbell. Excelsior. Heflin. Howard 17. Klondike. Missionary. U. S. D. A. No. 25.	Ananas de Guemene. Dunlap.	Aroma. Chesapeake. Fragaria americana. F. virginiana. Portia. Sample.	F. chiloensis. F. nilgerrensis.

Fragaria americana, although it differentiated fruit buds late, made a rapid growth in the late autumn, but failed to make a rapid growth in the spring. Chesapeake and *F. chiloensis* develop slowly during the autumn, bloom late in the spring, and ripen their fruit late in the season. Heflin, which differentiates fruit buds about midseason and makes only a moderate growth in the autumn, is among the first to bloom and to ripen in the spring. Klondike follows much the same trend in development as the Chesapeake during the autumn, but its growth is faster in the spring, and it ripens before the Chesapeake.

DISCUSSION

The results of these studies seem to indicate that the course of development of fruit buds in the strawberry family as a whole can not be definitely outlined. Each variety has a time and rate of development peculiar to itself. Although no study was made of the effect of cultural conditions upon the different varieties, it is believed that each would respond in a characteristic manner to changes in the environment. These varying responses are probably due to genetic differences that undoubtedly play a large part in the similar and dissimilar rates of development in the different varieties.

Owing to the varied parentage of the standard varieties, variations in development between different varieties under the same conditions are to be expected. With the utilization in breeding of plants obtained from remote sources, an even greater variation in response to the same set of conditions may be expected.

Fragaria nilgerrensis, native to northern India, differentiates fruit buds late in the fall. It may be expected that crossing our common varieties with such a species would enormously increase the possibilities of obtaining new varieties whose responses to environmental conditions would cover a far wider range of possibilities than that of our present varieties.

The time of fruit-bud differentiation in 1-year-old mother plants was found to be somewhat variable, particularly in Kalicene and other varieties differentiating rather early. This variability of fruit-bud differentiation in a single variety may be due to cultural practices and fertility of the soil. However, the influence of culture and of fertilizer applications on fruit-bud development needs further study.

SUMMARY

Indications of fruit-bud differentiation in the strawberry were found to extend over a considerable period before actual definite differentiation could be recognized.

Fruit-bud differentiation extended over a considerable period of time in some varieties; in others it occurred in a relatively short time.

Fruit-bud differentiation in all varieties and species studied under Maryland conditions, with one exception, was found to take place during September and early in October.

Fragaria nilgerrensis, a species native to northern India, did not differentiate fruit buds until November.

Rapidity and uniformity of subsequent development were found to vary to a considerable degree between the different varieties and species.

There is no absolute correlation between early differentiation of fruit buds, early blooming, and early ripening, or between late differentiation, late blooming, and late maturity. However, for many varieties this correlation actually existed.

FRUIT-BUD FORMATION IN EVERBEARING STRAWBERRIES¹

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INTRODUCTION

In the northern part of the United States everbearing strawberries fruit in the spring, in the summer, and in the fall until freezing weather occurs. This habit of continuous bearing seems to be the result of certain environmental conditions in some cases and of genetic characteristics in others.

Since Goff² found that fruit-bud differentiation took place in the Clyde, a spring-bearing variety, in September, reports of subsequent investigations have been confined entirely to the spring-bearing varieties. This neglect of the everbearing strawberry is attributed to its comparatively recent appearance in this country and to the fact that it is as yet of little importance in the fruit industry. However, with the recent introduction of the Mastodon variety more interest promises to attach to the growing of this type of strawberry.

The studies reported in this paper were undertaken to determine how and when fruit-bud formation takes place in certain varieties of the everbearing strawberries.

MATERIALS AND METHODS

The investigations were conducted at East Lansing, Mich.,³ during the summers of 1924 and 1925 and at the United States Plant Field Station, Glendale, Md., during the spring months of 1926 and 1927. The work at East Lansing was confined entirely to the Progressive, an everbearing variety originated by Harlow Rockhill of Conrad, Iowa. The plants used at East Lansing were grown on a well-drained sandy soil. The studies at Glendale included three varieties, all of which were originated by Rockhill. These plants were grown on a rich clay loam.

Crowns containing the buds for study were collected at intervals of 10 to 15 days throughout the experimental period in both localities. The usual methods of killing, fixing, and embedding as outlined by Chamberlain⁴ were followed. Allowing the buds to remain in a 10 per cent hydrofluoric acid solution for about three days made sectioning much easier, because the acid dissolved the crystals of calcium oxalate, which greatly interfered with sectioning. The sections were mounted on slides, and photomicrographs and camera-lucida drawings were made of them.

¹ Received for publication June 12, 1929; issued March, 1930.

² GOFF, E. S. INVESTIGATION OF FLOWER BUDS. Wis. Agr. Expt. Sta. Ann. Rpt. 17: 266-285, illus. 1900.

³ The results of the investigations reported herein pertain to work performed at East Lansing, Mich., and done in partial fulfillment of the requirements for the degree of master of science at the Michigan State College, East Lansing, Mich.

⁴ CHAMBERLAIN, C. J. METHODS IN PLANT HISTOLOGY. Rev. ed. 4, 349 p., illus. Chicago. [c1924.]

DEVELOPMENT OF THE CROWN

Vilmorin ⁵ says that the practical difference between the single and the perpetual-bearing strawberry can be traced back to an anatomical difference which consists in the production of flowering stems instead of runners from the axils of some of the leaves on the main stems. The investigations reported herein show that in the everbearing strawberry the growing point, which is located at the apex of the crown or runner, may differentiate into a fruit bud almost as soon as the elongating runner thickens into a crown, in some cases before roots appear. (Figs. 1, A and B.) However, a few leaves are usually produced in nearly all new-runner plants of everbearing strawberries before the growing point develops into a fruit bud. After a growing point of a crown has differentiated into an inflorescence there appears in the axils of many of the earlier formed leaves, possibly of all of them, meristematic tissue which may differentiate immediately into an inflorescence. However, there are usually a few or several leaves produced first which form a new crown. Sooner or later the growing point of this crown differentiates into an inflorescence, after which no further elongation of the crown can occur. Similarly, other crowns arise on those already developed, and thus in the course of time an old crown becomes very much branched, as is shown in Figures 4, C and D; 2, A, B, and C.

Everbearing strawberries produce a spring crop at about the same time that the spring-bearing kinds are fruiting. Following the spring crop there seems to be a period during which only a few ripe berries are produced. However, during this time, late May to early July, everbearers produce most of their runners. (Fig. 3.) Such everbearing varieties as Progressive, Rockhill, and Americus are not prolific runner producers. Apparently at the time the spring crop is ripe, or just following it, runners rather than branch crowns are developed in the axils of the leaves. Instead of the formation of only a few branch crowns and the production of runners in the axils of all the leaves, as occurs in the spring-bearing plants, many branches are formed and only a few runners are produced.

FRUIT-BUD DIFFERENTIATION

During the summers of 1925 and 1926, studies in Michigan showed that differentiation of fruit buds in the Progressive variety occurs throughout the summer. However, there was found to be a break in the continuous production of fruit; this break occurred immediately following the maturity of the spring crop. Before these observations were made the interrupted production between the spring crop and the summer and fall crop was assumed to be due to a corresponding break in the continuity of fruit-bud differentiation.

In order to determine how early in the spring fruit-bud differentiation takes place, studies were conducted at the United States Plant Field Station during the spring months of 1926 and 1927 with three varieties, Progressive, Rockhill, and Americus. Only a few early stages in fruit-bud formation were found until late in May. Those early stages which were found in April and early May may have been differentiated the previous fall. It seems apparent that a break

⁵VILMORIN, H. DE. PERPETUAL STRAWBERRIES. *Roy. Hort. Soc. Jour.* 22: 311-326, illus. 1898-99.

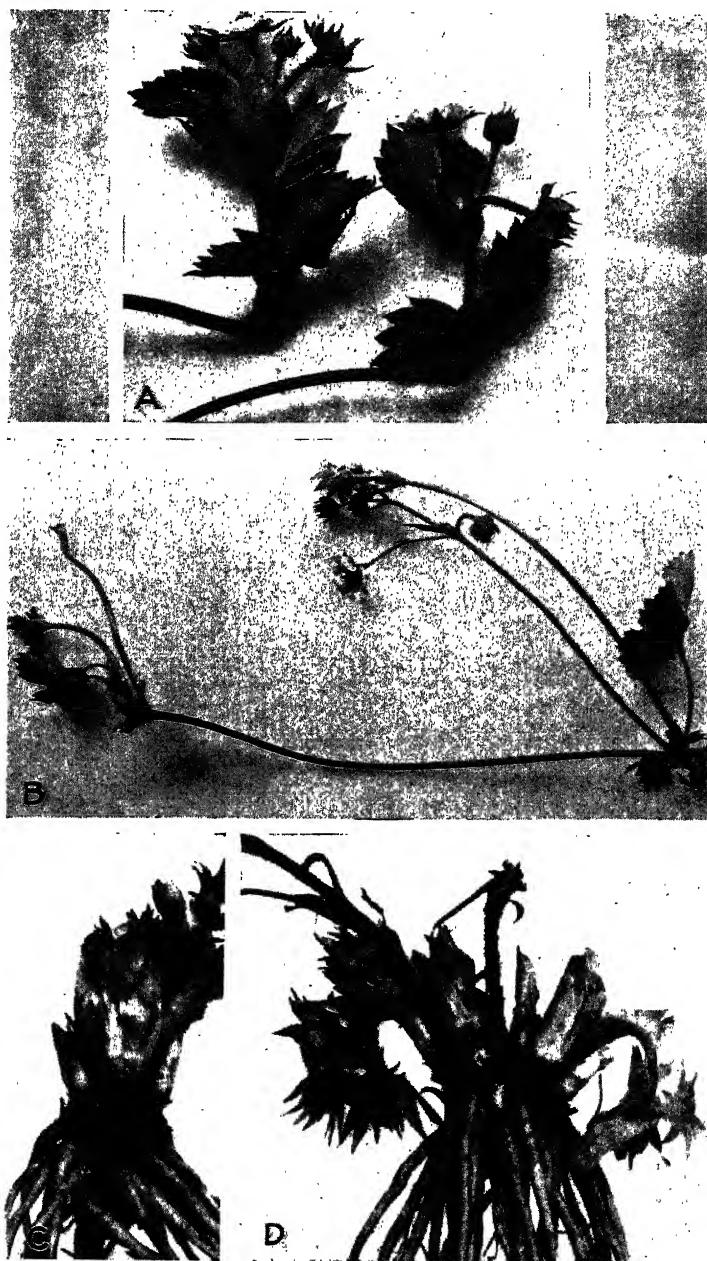


FIGURE 1.—A, Runner plants of U. S. D. A. No. 447 strawberry, with flowers open but no roots appearing. July, 1927. B, Runner plants of U. S. D. A. No. 400 strawberry beginning to take root and having flower stalks with open blossoms. July, 1927. C, Young plant of the Progressive strawberry, showing buds on the crown developing in the axils of the leaves, which have been removed. Photographed January 11, 1929. D, Runner plant of the Progressive strawberry which had taken root in June, 1928, showing old fruit stalks attached below the region where new branching is taking place. Photographed January 11, 1929.

occurs in the continuity of fruit-bud differentiation in April and early May, a period when the fruit buds differentiated the previous fall are developing into fruit clusters. Figures 4, 5, and 6 show photomicro-

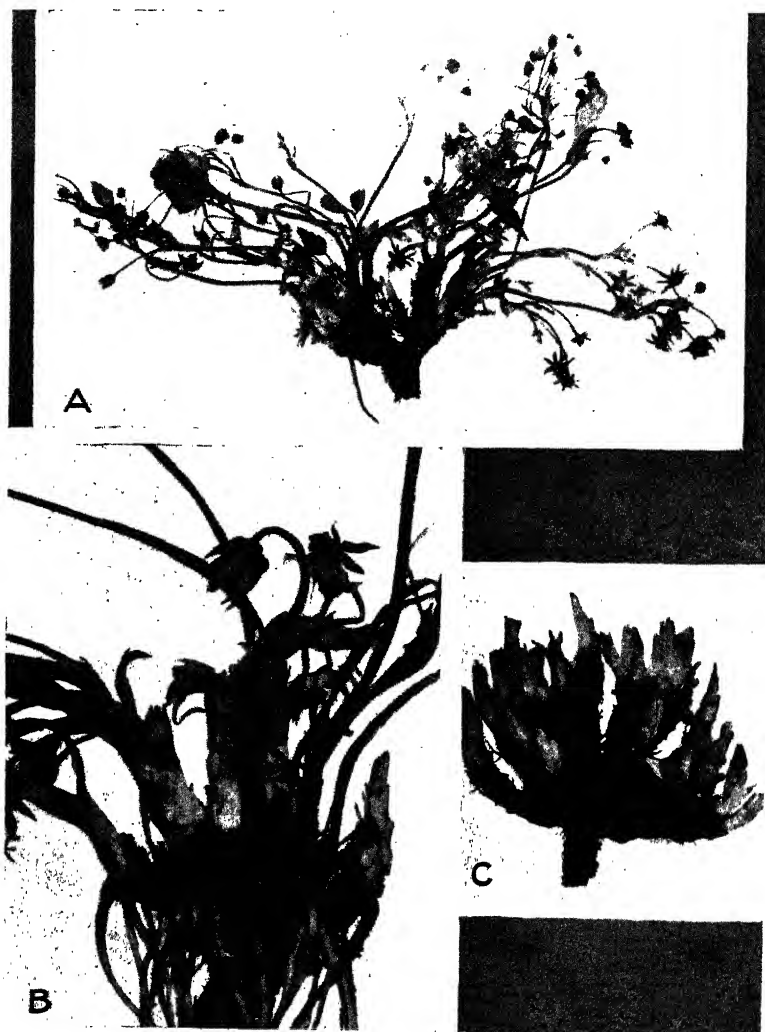


FIGURE 2.—A, Old plant of the Progressive strawberry, with old fruit stalks still attached. Photographed January 11, 1929. B, An old Progressive strawberry plant, showing old fruit stalks attached lower on the crown and new flower stalks terminating the new branches. Photographed January 11, 1929. C, Two-year-old Progressive strawberry plant from which leaves and roots have been removed in order to show the branched crown

graphs of the early stages in fruit-bud development, in both spring and fall, in the three varieties studied.

The fruit that ripens in May and early June develops from buds which apparently differentiated the previous fall, and the fruit that ripens in July develops from fruit buds differentiated late in May and

in June. Because of little or no differentiation in April, little or no fruit is produced in June and early July. Runner production is high during this time, but gradually becomes less, with fruit production increasing as the summer advances.

DISCUSSION AND SUMMARY

After producing a spring crop of fruit, strawberries of the everbearing type send out a few runners from the axils of their leaves. Instead of continuing runner production over an extended period, like the spring-fruited type, everbearers may send out a flower stalk from



FIGURE 3.—A seedling clon of everbearing strawberry producing fruit buds on many runner plants and on some before they had rooted. July 13, Osage, Iowa

the leaf axil, though more often they produce very short branches, the growing point of each of which soon differentiates a fruit bud. As a result, fruit is produced throughout the summer from a crown which gradually becomes more and more branched, the process continuing until the cold of winter checks growth.

The almost entire absence of developing flower stalks over a period of three or four weeks immediately following the spring crop, together with the existence of favorable temperatures and long days, are suggested as the causes of the unusual activity of everbearing strawberries in producing runners, new branches, and differentiating fruit buds all at this time.



FIGURE 4.—A, Fruit bud of the Progressive strawberry, showing secondary flowers differentiating on May 25, 1926, at Glendale, Md. B, Fruit bud of the Progressive strawberry in the process of differentiation on June 27, 1926, at Glendale, Md. $\times 90$



FIGURE 5.—A, Fruit bud of the Rockhill strawberry in process of differentiation on June 8, 1926, at Glendale, Md. B, Fruit-bud differentiation just beginning in the Rockhill strawberry on October 7, 1926, at Glendale, Md. $\times 90$



FIGURE 6.—Fruit bud of the Americus strawberry, showing secondary as well as primary flowers in process of differentiation on June 8, 1926. $\times 90$

EFFECT OF LEAF RUST (*PUCCINIA TRITICINA* ERIKS.) ON YIELD OF WHEAT¹

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INTRODUCTION

The leaf rust of wheat (*Puccinia triticina* Eriks.) is more or less prevalent throughout the wheat-growing areas of the world. It very often so infects wheat that all the leaves and even the glumes are covered with uredinia. Opinions concerning the importance of this rust in wheat production, however, have varied considerably. Eriksson and Henning (4)³ concluded that, in Sweden, leaf rust is rather innocuous because it occurs only on the leaves of its hosts. Carleton (2, p. 40-41) in the United States stated that—

the orange leaf rust, as a rule, does very little, if any, damage to wheat, even during periods when it is quite abundant. * * * Occasionally, under certain conditions and in certain localities, considerable injury may ensue if the rust occurs much in advance of harvest.

McAlpine (10, p. 64), in 1906, reported "there are only two kinds of rust in wheat in Australia, the positively injurious *Puccinia graminis* and the comparatively harmless *P. triticina*, because it does not pinch and shrivel the grain like the other." However, in Australia, Cobb (3), in rust studies published from 1890 to 1894, recognized leaf rust as being destructive to wheat varieties commonly grown there at that time. According to Klebahn (9), leaf rust is one of the most prevalent diseases and occasionally most serious, causing much damage in the Province of Brandenburg, Germany. Grove (5, p. 263) stated that "the Brown Rust of Wheat [*P. triticina*] has been frequently so abundant in this country [Britain] in its uredo-stage as to cause great loss." Such statements are representative of the diverse opinions held by pathologists concerning the effect of the leaf rust on the yield of wheat.

In the United States, the Plant Disease Survey of the Bureau of Plant Industry, United States Department of Agriculture, was started in 1917 to obtain information concerning various plant diseases. In the summary of data on cereal crops, Haskell⁴ made the following statements about leaf rust:

The economic importance of this rust has been questioned by many pathologists. It is maintained that the affection of the leaves, coming on rather late in the

¹ Received for publication July 24, 1929; issued March, 1930. Published with the approval of the director as a contribution from the department of botany, Purdue University Agricultural Experiment Station, La Fayette, Ind. Cooperative investigations between the Purdue University Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

² The writer wishes to express his thanks to C. R. Ball and H. B. Humphrey, of the Office of Cereal Crops and Diseases, for many helpful suggestions and criticisms, and his appreciation of the assistance rendered by Miss Dorothy M. Thompson and Leroy E. Compton in the course of these investigations.

³ Italic numbers in parentheses refer to "Literature cited," p. 446.

⁴ HASKELL, R. J. SUMMARY OF PLANT DISEASES IN THE UNITED STATES IN 1918. IV. DISEASES OF CEREALS AND FORAGE CROPS. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 4: 119-159, illus. 1919. [Mimeographed.] (See p. 131.)

season, as it usually does, does not materially reduce the size or number of kernels. After the kernels are well formed it is said that the leaves are not necessary for full development of the grain so long as the culms remain green. In 1917, collaborators were asked in a questionnaire if they considered the disease of economic importance and replies from Pennsylvania, Maryland, North Carolina, South Carolina, Tennessee, and Indiana were in the affirmative. Collaborators in most of the other States indicated that usually it was not of importance. In Oregon it was said to be of importance on certain varieties only.

Melchers (12) reported that in Kansas, during 1917, leaf rust was abundant and that careful observation indicated that no other factor could be responsible for the poor quality and low yields. The yield of one field was reduced 38 per cent.

In 1918, an investigation of the leaf rust of wheat was started as a cooperative project between the Purdue University Agricultural Experiment Station and the Office of Cereal Crops and Diseases of the Bureau of Plant Industry, United States Department of Agriculture. On account of the differences in opinion and the lack of accurate information concerning the effect of leaf rust on the yield of wheat, a series of experiments for the purpose of comparing rusted and rust-free wheat was started in 1921 as one phase of the investigation.

EFFECT ON SUSCEPTIBLE VARIETIES

GREENHOUSE STUDIES

In 1921, Red Fern, a spring wheat, and Mediterranean, a winter wheat, both very susceptible to leaf rust, were used. They were grown in the greenhouse at the Purdue University Agricultural Experiment Station in 6-inch pots, one plant to a pot. Six plants of Red Fern and five of Mediterranean were inoculated 40 days after planting, when the tillers were well developed. An equal number of plants were left uninoculated as checks (C). The resulting infection of the inoculated ⁵ plants was slight, averaging 15 per cent for Red Fern and 10 per cent for Mediterranean. These infection percentages represent the actual amount of infection compared with the amount possible. No infection occurred on the check plants. The rusted series were reinoculated at intervals of 25, 18, 16, 17, and 24 days with average infections of 15, 30, 40, 65, and 85 per cent resulting. The maximum infection was obtained when the plants were slightly past blossoming. A slight infection developed on the checks, reaching 35 per cent following blossoming.

The plants of the rusted series (R) were slightly stunted (fig. 1) and developed more tillers than the checks (C). Many tillers of the rusted plants (R), however, did not develop heads, and the heads produced were somewhat later in maturing than those of the checks (C).

The heads of each plant were harvested and threshed separately. Table 1 gives the results obtained. The rusted plants of Red Fern produced 15.5 per cent fewer heads than the checks, while Mediterranean showed a reduction of 12.1 per cent. There also was a decided reduction in the number of kernels per head. Red Fern averaged 7.9 kernels per head for the rusted compared with 12.1 for the checks. Mediterranean averaged 8.8 kernels for the rusted compared with 15.7 for the checks. While a large part of the reduction in yield was due to the fewer kernels produced, a portion was due to reduction in weight of the kernels, as is shown by the average weight per kernel.

⁵ Throughout this paper such inoculated series are referred to as the rusted series (R).

In both the check and the rusted plants the grain showed shriveling. While practically all the grain from the rusted plants was shriveled, the check plants showed 43 per cent of shriveled kernels.

In this experiment, the plants were infected only slightly to moderately during their earlier development. Even after heading, the



FIGURE 1.—Plants of Red Fern wheat: A, Moderately rusted from tillering to maturity and showing stunting and fewer heads than B, which is slightly rusted

infection never reached 100 per cent. Some infection, however, occurred during a considerable portion of the development. Such a condition probably seldom occurs in the field in the northern part of the United States, but in the South such a development of rust may occur occasionally.

TABLE 1.—Effect of infections of various intensities and durations by the leaf rust on yield of wheat in the field and in the greenhouse at La Fayette, Ind.

[The capital letters in column 3 indicate different treatments discussed in the text. T=trace]

Year	Variety	Treatment	Amount of rust ^a	Stage of growth	Units	Heads	Kernels	Average kernels per unit	Reduction in number of kernels	Yield (total)	Average	Reduction in weight	Average weight per kernel
			P. ct.		No.	No.	No.	No.	P. ct.	Gm.	Gm.	P. ct.	Mg.
1921	Red Fern	C	0-35	Tillering	6	71	862	143.66 ±13.5		24.3	4.05 ±0.38		28.3
1921	do.	R	15-85	do.	6	60	475	79.16 ±13.24	44.9	10.4	1.73 ±.34	57.2	21.9
1921	Mediterranean	C	0-35	do.	5	74	1,164	232.8 ±16.53		26.7	5.35 ±.053		22.9
1921	do.	R	10-85	do.	5	65	576	115.2 ±14.0	50.5	9.6	1.92 ±.286	63.3	16.7
1922	Fulcaster	CA	0-5	Heading	12	33	3,883	323.6 ±21.85		139.5	11.6 ±.736		35.9
1922	do.	RB	75-100	Tillering	6	4	90	15.0 ±2.54	95.4	1.9	.32 ±.096	97.4	21.1
1922	do.	RC	50-100	Shooting	12	13	409	34.1 ±7.61	89.5	12.3	1.02 ±.196	91.3	30.1
1922	do.	RD	100	Boot	9	53	1,342	149.1 ±.52	53.9	47.9	5.32 ±.189	54.3	35.6
1923	do.	C	0-5	Blossoming	438	99				73.76	1.94		
1923	do.	R	75-100	do.	448	121				70.12	1.46	24.7	
1925	Michigan Amber	C	0-1	do.	8	239	2,515	314.37 ±14.61		90.18	11.27 ±.513		35.8
1925	do.	R	100	do.	8	234	2,009	251.37 ±14.07	20.1	59.88	7.48 ±.173	33.5	29.8
1927	do.	CA	0-1	Boot	15	95	2,183	145.5 ±4.76		72.14	4.81 ±.173		33.0
1927	do.	RA	75-100	do.	15	95	1,910	127.3 ±5.09	12.5	45.31	3.02 ±0.73	37.2	23.7
1927	do.	CB	0-T	Blossoming	5	241	3,285			120.39	24.08 ±1.03		36.6
1927	do.	RB	90-100	do.	5	246	2,963		9.8	87.68	17.54 ±0.56	27.2	29.6
1927	Illinois No. 1	CC	75-100	Ripening	80					11,967.0	149.5 ±2.09	24.2	
1927	do.	DC	T-35	do.	80					15,794.0	197.4 ±3.18		
1927	do.	CD	75-100	do.		150	3,681	24.5 ±.46	18.9	101.1		25.8	27.5
1927	do.	DD	T-35	do.		150	4,538	29.9 ±.49		136.3			30.0
1927	Trumbull	CE	75-100	do.	80					22,742.0	284.3 ±3.38	11.1	
1927	do.	DE	T-35	do.	80					25,575.0	319.7 ±3.66		

^a The minimum rust intensity occurred at the stage given in column 5 and developed later to the maximum.^b Number of pots; one plant grown in a 6-inch pot.^c Number of pots; four plants grown in a 6-inch pot.^d Number of plants.^e Number of rows in the greenhouse bench.^f Number of pots; two plants in each rusted and two as checks.^g Number of rod rows in field plots.^h Rod rows in field plots dusted with sulphur to prevent rust development.ⁱ Sample of 150 heads selected from dusted and rusted plots.

In 1922 an experiment was conducted to determine the effect of leaf-rust infection at different stages of development. A strain of Fulcaster (C. I.⁶ No. 4892), very susceptible to leaf rust, was used. Four plants were grown in each 6-inch pot in order more nearly to approach field conditions. The pots of wheat were divided into four lots. Lot A, comprising 12 pots, was used as check. (CA, Table 1.) A slight infection, about 5 per cent, developed following heading. Lots B, C, and D were rusted plants. (RB, RC, and RD, Table 1.)

⁶ C. I. indicates accession number, Office of Cereal Crops and Diseases.

Plants of lot B, consisting of 6 pots, were inoculated as soon as they were well tillered. The infection was severe, averaging 75 per cent. The plants of this lot were reinoculated at intervals as new leaves developed, and an infection of 100 per cent was maintained for most of the development of the plants. Plants of lot C, consisting of 12 pots, were inoculated when they were starting to shoot. The initial infection was 50 per cent. These plants were reinoculated at intervals and an infection of 100 per cent was obtained and maintained to maturity. Plants of lot D, consisting of 9 pots, were inoculated when the first indication of heading was evident. At this time all the leaves were practically fully developed. An infection of 100 per cent was obtained.

Table 1 also gives the effects on yield for 1922 in lots A to D of Fulcaster wheat. The early infections of lots B and C resulted in a marked reduction in the number of heads developed. While the check pots averaged 6.9 heads per pot, lot B averaged only 0.6 and lot C only 1.1 heads per pot. Lot D, inoculated when the heads were showing in the boot, averaged 5.9 heads. The plants subjected to the earlier infections were stunted. Even those culms which produced heads were shorter than those of the check plants. The lower leaves of the rusted plants also died considerably in advance of those of the check. There was very little difference in height between the late-infected (lot D) and the check plants. The leaves of the rusted plants, however, died more rapidly than those of the check. Ten days following inoculation, when uredinia were just showing, the upper three leaves of the rusted plants were green and the lower two were yellowish and dying at the tips. The corresponding five leaves of the check plants were entirely green. Thirteen days later the two upper leaves of both the rusted and the check plants were still green. About one-half of each of the next two lower leaves of the rusted plants was dead, while only the tips of the corresponding leaves of the check plants showed indications of dying. The lower leaves of the rusted plants were entirely dead, while the terminal halves of those of the check plants were yellowish. Thirteen days later only the basal portions of the two upper leaves of the rusted plants were still green and the three lower leaves were entirely dead. Except for the tips, which were yellowish or dying, the three upper leaves of the check plants were green. From one-third to seven-eighths of each of the two lower leaves was yellowish or dead. Six days later all the leaves of the rusted plants were dead, but the upper leaf of the check plants was green and about one-eighth to one-half of each of the lower leaves still showed green tissue. The plants were mature 10 days later.

The straw from the 12 check pots weighed 438.5 gm., an average of 36.5 gm. per pot. The straw from the 6 pots of early-inoculated wheat (lot B) weighed 64.5 gm., or an average of 10.7 gm. per pot and a reduction of 70.6 per cent. The straw from the 12 pots of the next inoculation (lot C) weighed 120.5 gm., an average of 10 gm. per pot and a reduction of 72.6 per cent. The straw from the 9 pots of the late inoculation (lot D) weighed 219.7 gm., an average of 24.4 gm. per pot and a reduction of 33.1 per cent.

The grain produced by the earlier infected plants (lots B and C), was not so plump as that produced by the check plants, as is shown by the average weight per kernel in Table 1. The early-inoculated lot (B) had 71.4 per cent of shriveled kernels and the next lot (C) had

71.7 per cent, as compared with 20.2 per cent in the case of the check plants. The late-inoculated lot (D) produced kernels of practically the same average weight as those produced by the check plants and contained fewer (13 per cent) shriveled kernels, because a large number of shriveled kernels had been produced by one plant of the check.

The early infections exerted a very pronounced effect upon the development of the wheat plant. The large losses in yield were due to a reduction in the number of heads and kernels produced, although the average weight per kernel also decreased very markedly. Late infection brought about a reduction in yield largely by reducing the number of kernels produced. There was also some reduction in weight per kernel, which, however, is obscured by the shriveling in one of the check plants.

In 1923 an experiment was conducted in which Fulcaster wheat was inoculated still later in its development. The results are shown in Table 1. Thirty pots were sown with the Fulcaster variety. Four plants were grown in each pot. Plants in half of the pots were inoculated (R) with rust when the heads had started to blossom. An infection of 75 to 100 per cent was obtained. The check plants (C) developed a slight amount of rust (5 per cent) in some cases. Unfortunately, sparrows entered the greenhouse when the plants were about ready to harvest and ruined part of the plants. These were eliminated from the experiment, and the yield, consequently, is based upon the average yield per plant, rather than per pot as in previous years. On account of lack of assistance, the plants were not harvested separately, and the kernels were not counted. The average number of heads per plant in the check pots was 2.6 and in the rusted 2.5. As the plants were not inoculated until the heads were in bloom, no effect on head production was to be expected. The reduction in yield, although still considerable, was much less than when infection took place earlier.

In 1924 a similar experiment was undertaken. Mildew developed on the plants during the winter, however, and the experiment was discontinued.

In 1925 a strain (29-1-1-1) of the Michigan Amber variety, which had been found resistant to powdery mildew (*Erysiphe graminis*) and highly susceptible to leaf rust, was used. In order to approach field conditions as closely as possible, the wheat was sown in rows in the greenhouse bed. Sixteen 5-foot rows were spaced 6 inches apart, and the kernels were dropped approximately 1 inch apart in the rows. The soil was ordinary well-mixed field soil taken from plots back of the experiment station. The plants were inoculated on April 17, when the heads were well developed and in early bloom. The rows were covered with a muslin cage with a partition separating them into two series of 8 rows each. Each series was heavily atomized, and one (R) was thoroughly dusted with urediniospores of leaf rust by shaking heavily rusted plants over them. The other series of eight rows was left uninoculated. The muslin cover over each cage was then thoroughly wet. The inoculation was made in the late afternoon, and the next morning all covers were removed. Uredinia were first noted on the sixth day, and in 10 days an infection of 100 per cent had developed on the inoculated plants (R). The check (C) rows showed no rust at this time. Although care was taken in watering to prevent wetting the foliage, a slight infection of about 1 per cent developed

before maturity on the lower leaves of the plants in the check rows. On May 20, 33 days after inoculation, only the upper or flag leaf of the infected plants was still alive. The check plants had two or three of the upper leaves green and in good condition. A week later when the heads of both rusted and check plants were showing signs of ripening, all the leaves of the rusted plants were dead, while one or two upper leaves of the checks were still in a good condition. The rows were all harvested on June 6. A few of the flag leaves of the check lots were still partly green, although the heads were ripe.

On account of the difficulty of separating the material by plants, the wheat was harvested by rows. There was practically no difference in the number of heads produced. (Table 1, 1925.) Most of the reduction in yield was due to the fact that fewer seeds were produced by the rusted plants, although there was also a reduction in the average weight of the kernels. There was very little shriveling, the check having about 3.1 per cent, and the rusted plants a little over twice as much, or 7.7 per cent of shriveled kernels.

In 1926 the previous experiment was repeated, using a heavily fertilized soil (greenhouse compost). During the winter the growth was so rank that considerable lodging and irregular overshadowing and development occurred. It was evident that the rows could not be divided in a comparable way, and the experiment was discontinued.

In 1927 studies were made in the greenhouse with wheat in pots (A) and in rows in the bench (B). Fifteen 6-inch pots, each containing four plants of Michigan Amber (29-1-1-1), were used in the pot experiment. The plants were inoculated when the heads were just emerging from the boot. In order to reduce as much as possible the variation due to differences in environmental conditions, each pot was divided into two parts with muslin, each part containing two plants. Two of the plants of each pot were inoculated on May 1 (RA, Table 1), and the other two served as checks (CA, Table 1). Twelve days later the inoculated plants showed 75 to 100 per cent of rust. The check plants showed an occasional uredinium, and before maturity about 5 per cent of rust had developed on the lower leaves, representing an average of 1 per cent for the whole plant in the checks. At this time the uppermost leaf of each infected plant was slightly yellowish at the tip, while the three leaves below were entirely yellowish green. The three upper leaves of the check plants were green and one leaf below was yellowish. Twelve days later (May 24) the flag leaves of the infected plants were yellowish green and all the other leaves were dead. The three upper leaves of the check plants were green and the next ones below were dead. Fourteen days later (June 7) all the leaves of the infected plants were dead, the heads were nearly all ripe, and the straw was yellow. A few of the flag leaves of the check plants were yellowish green, while the rest were dead. Most of the heads were ripe. The straw showed a decided purpling.

Both the rusted plants and the check plants had the same number of heads. (CA and RA, Table 1, 1927.) However, there was not only a decided reduction in the number of kernels produced (12.5 per cent), but also considerable of the reduction in yield was due to a decrease in weight of the kernels, as is shown by a comparison of the average weights per kernel.

In 1927 the Michigan Amber variety (29-1-1-1) was sown also in the greenhouse bench in rows 4 feet long and 6 inches apart. The

seeds were spaced about an inch apart in the row. The plants were inoculated when the heads were fully developed and after blossoming had started. As in 1925, the rows were divided into two groups of 5 rows each by muslin cages, when they were inoculated. Five rows were inoculated (RB) and five served as checks (CB).

Fifteen days following inoculation the plants in the inoculated rows showed 90 to 100 per cent of rust. The check plants showed no infection to a trace. Twenty-seven days after inoculation the flag leaves of the infected plants were still alive, although slightly yellowish green; the other leaves were dead, except for an occasional leaf just below the flag leaf, which was still alive but yellow. At this time the two upper leaves of the check plants were entirely green, the third leaf from the top was yellowing at the tip, and the lower leaves were dead. Thirty-eight days after inoculation practically all of the infected leaves were dead. The stalks and heads of the infected plants were yellowing. The upper leaves of the check plants were about half green, the heads were yellowish, and the stalks were a pronounced purple. Nine days later both the rusted and the check plants were ripe. The straw of the rusted plants was a dull yellow, while that of the checks plants showed considerable purpling.

There was practically no difference in the number of heads produced by the rusted (RB) and check (CB) rows. (Table 1, 1927.) While part of the reduction in yield was due to the fewer kernels produced by rusted plants, most of it was brought about by reduction in weight per kernel. Although part of this was due to an increased production of shriveled kernels, 3.1 per cent in the check and 8.6 per cent in the rusted plants, most of it may be accounted for by the fact that the kernels were slightly smaller and consequently lighter. There was considerably more yellow berry in the grain from rusted plants, 32.8 per cent for the rusted as compared with 16.4 per cent for the check plants. The weight of the air-dried straw from the five check rows was 305 gm. and from the rusted rows 270 gm., a reduction of 11.4 per cent.

FIELD STUDIES

Sulphur has proved to be a very effective fungicide for preventing the germination of urediniospores of the leaf rusts.⁷ Kightlinger (7) has reported experiments showing that sulphur applied as a dust was effective in controlling crown rust of oats in the field. Kightlinger and Whetzel (8) and Bailey and Greaney (1) reported the successful use of sulphur dust to control leaf rust of wheat in the field.

During 1925 and 1926, attempts were made to obtain data on losses produced by leaf rust under field conditions. The use of sulphur fungicides as sprays and dusts resulted unsuccessfully because of the slight amount of rust present in the experiment station plots in both years.

In 1927 two varieties of wheat—Trumbull, a winter wheat, and Illinois No. 1, a spring wheat—were sown on the agronomy farm at La Fayette, Ind., where leaf-rust epidemics are more certain to occur. Each variety was sown in twenty-five 18-foot rows, 1 foot apart, in each of eight plots. Each plot was sown as follows: 1 row for border;

⁷ Data obtained in studies for the degree of master of science in agriculture in the department of botany, Purdue University Agricultural Experiment Station, and presented, in an unpublished thesis, by Leroy E. Compton, assistant pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, 1926.

10 rows dusted with sulphur (Kolodust); 3 rows to serve as protection against dust drift; 10 rows not dusted; 1 row of border. This provided eight 10-row plots for dusting and eight 10-row plots not dusted for each variety, besides guard and separation rows.

The spring variety, Illinois No. 1, was dusted six times between May 25 and July 5. Leaf rust was first noted on May 31, when the plants were shooting. At this time a trace of rust occurred on the undusted rusted check plants and none on the dusted ones. On June 23 the plants of this variety were blossoming, and 25 to 50 per cent of rust occurred on the undusted rusted checks and a trace to 5 per cent on the dusted plants. On July 8 the kernels were about two-thirds formed and 50 to 100 per cent of rust had developed on the undusted rusted checks (Table 1, 1927, CC) and a trace to 35 per cent on the dusted plots (DC). On July 16 the wheat was ripening and the undusted rusted checks showed 75 to 100 per cent of rust (CC) and the dusted still a trace to 35 per cent (DC).

Leaf rust became destructive on Illinois No. 1 at an earlier stage in its development than in the case of Trumbull. A moderate to heavy infection from blossoming to maturity resulted in an average yield of only 149.5 gm. per row, or 14.95 bushels per acre, from the undusted and rusted checks (Table 1, 1927, CC), whereas the dusted plots produced an average yield of 197.4 gm. per row, or 19.74 bushels per acre (Table 1, 1927, DC). The reduction caused by rust was 4.79 bushels per acre, or 24.2 per cent.

A sample of 150 heads was selected at random from both the undusted and rusted checks and the dusted plots of Illinois No. 1. These heads were carefully threshed by hand, and the kernels were counted for each head. As shown in Table 1 (1927, CD and DD), the total reduction in yield agreed rather closely with that in the yield from the entire eight 10-row plots (Table 1, CC and DC). This study shows that the reduction in yield was due largely to the fact that the undusted and rusted plants produced fewer kernels.

The Trumbull winter wheat (fig. 2) was given five dustings between May 25 and June 27. On May 31 the wheat was in head, and a trace to 10 per cent of rust was noted in the undusted check plots, while none was present in the dusted plots. On June 23 the wheat was considerably past blossoming, and 50 to 100 per cent of rust had developed on the undusted rusted checks, whereas only a trace to 10 per cent occurred on the dusted plots. (Fig. 2.) At that time practically all of the lower leaves of the rusted plants were dead and the upper leaves were yellowish. Most of the lower leaves of the dusted plants were dead, but the upper ones were still green. On June 29 the wheat was ripening and the undusted rusted check plants showed 75 to 100 per cent of rust (Table 1, 1927, CE) and the dusted a trace to 35 per cent (Table 1, 1927, DE). All the leaves of the rusted plants were dead. Most of the upper leaves of the dusted plants were still alive but were yellowish. Both undusted and dusted plots were harvested July 5, a foot being trimmed from each end of the rows and the yield obtained in grams for a 16-foot row.

The rust on Trumbull did not become severe until it was well past blossoming, and it reached a maximum only a short time before maturity. The average yield of the undusted rusted check plots was 284.3 gm. per row, or 28.43 bushels per acre (Table 1, 1927, CE),

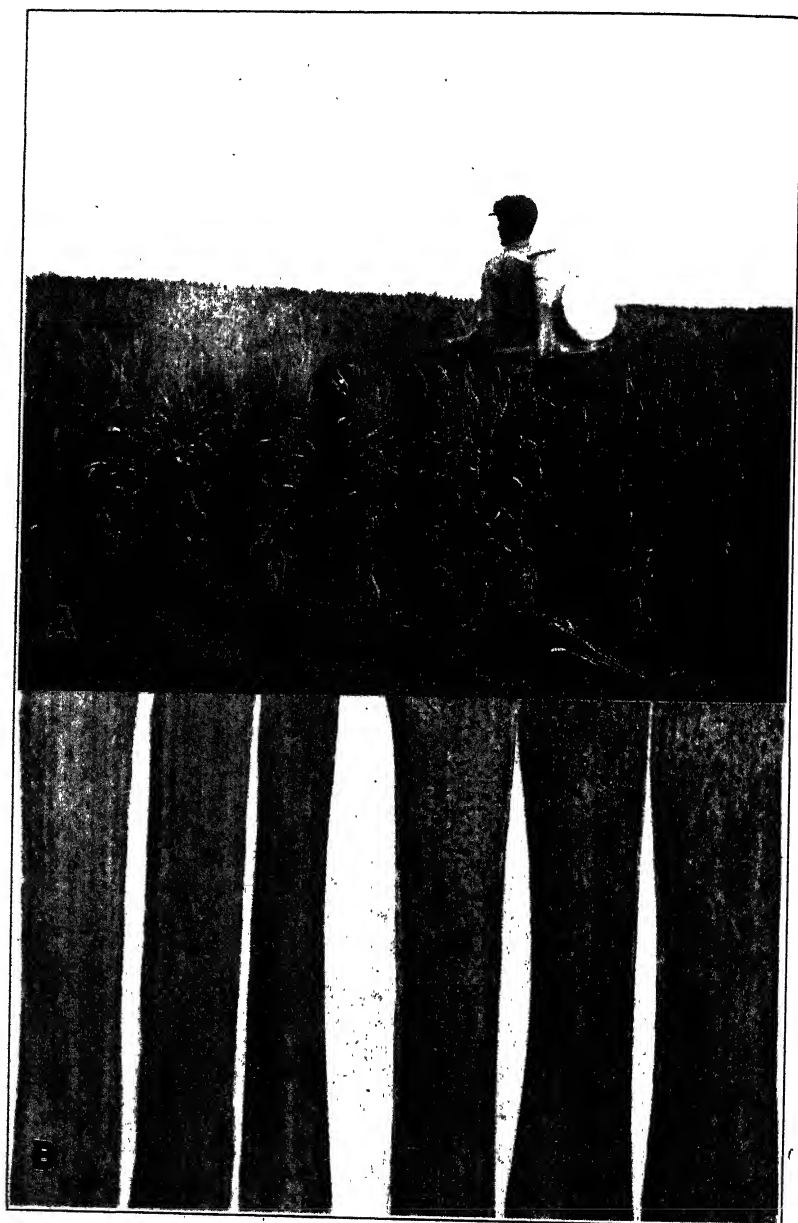


FIGURE 2.—A, Dusting plots of Trumbull wheat with sulphur to control leaf rust; B, three clean leaves from sulphur-dusted plots; C, three rusted leaves from plots not dusted and heavily rusted

whereas the average of the dusted plots was 319.7 gm. per row, which is equivalent to 31.97 bushels per acre (1927, DE). The reduction in yield was 3.54 bushels, or 11.1 per cent.

As is evident, the rust was able to develop on the undusted plants to a considerable extent, while it was largely prevented from developing on those which were dusted. The former, therefore, are comparable to the rusted plants in the greenhouse studies, and the latter serve as checks in a comparison of the effects of the rust upon yield.

EFFECT ON A RESISTANT VARIETY

During the winter of 1926-27 a study was made in the greenhouse to determine the effect of infection on a resistant variety. Ten pots of the Webster variety (C. I. 3780), four plants to a pot, were used in the greenhouse in a manner similar to that employed with Michigan Amber. Webster is very resistant to physiologic form 5, which was used for inoculation (11). On May 1, two plants of each pot were inoculated. At this time the heads were just emerging from the boot. On May 12 infection was indicated on the inoculated plants by numerous small necrotic flecks. Approximately as much infection occurred as in the susceptible Michigan Amber (Table 1, 1927, CA and RA), but the infected cells of the host were soon killed and the rust did not sporulate. The flag leaf was green and apparently in good condition. The two leaves next below showed considerable yellowing. The next lower leaf was dead. On the check plants the three upper leaves were in good condition. The fourth leaf from the top showed considerable yellowing. On May 24 virtually all the leaves of the inoculated plants were dead. The two upper leaves of the check plants were green, the third leaf from the top was yellow and dying, and the fourth was dead. On June 7 the rusted plants were ripe, while the check plants still showed a slight amount of green color in the leaves. The heads and kernels were apparently mature.

All the plants were ripe on June 15 and were then harvested. The 20 check plants produced 1,040 kernels, weighing 41.32 gm., while the rusted ones yielded 1,130 kernels, weighing 36.58 gm. On the basis of total production, the rusted plants showed an increase of 8.6 per cent in number of kernels. However, it is evident that infection had a decided effect on the weight of grain, the total yield of the infected plants being reduced 11.4 per cent. The average weight per kernel from the check plants was 39.6 mg. and from the rusted plants 32.3 mg. The reduction in yield for the correspondingly inoculated susceptible Michigan Amber was 37.2 per cent.

From this one experiment it would appear that the infection of resistant varieties may result in a decided reduction in yield, the effect being largely produced by a reduction in size and weight of the kernels.

EFFECT ON KERNEL FORMATION

As can be seen from Table 1, the reduced yield resulting from rust infection was due in part to the production of fewer kernels and in part to a reduction in the weight of the individual kernels. A study of the number of kernels produced per spikelet by rusted plants and by check plants, respectively, was made to determine in what part of the head the reduction in number occurred. The heads from a representative sample of both rusted and check plants were threshed by

hand. The number of kernels for each spikelet in each head was recorded, as well as the position of the spikelet in the head. In this way 21,769 spikelets from 1,331 heads were studied (Table 2) from material obtained during the four years of the investigation, 1921, 1922, 1925, and 1927.

TABLE 2.—*Effect of the leaf rust of wheat on the number of kernels produced per spikelet*

[See Table 1 for rust intensities and duration]

Year	Variety	Condition of plant	Number of heads	Number of spikelets producing—						Total kernels
				0 kernel	1 kernel	2 kernels	3 kernels	4 kernels	5 kernels	
1921.....	Mediterranean.....	(Check.....	74	614	435	348	11	0	0	1,164
		(Rusted.....	65	724	318	129	0	0	0	576
1922.....	Fulcaster.....	(Check.....	47	293	132	242	288	101	5	1,909
		(Rusted.....	47	554	134	142	115	16	2	837
1925.....	Michigan Amber.....	(Check.....	59	376	212	238	19	0	0	745
		(Rusted.....	62	406	202	183	18	0	0	622
1927.....	do.....	(Check A.....	95	615	286	547	259	4	2	2,183
		(Rusted A.....	95	676	383	583	119	1	0	1,910
1927.....	do.....	(Check B.....	241	1,645	668	825	293	22	0	3,285
		(Rusted B.....	246	1,768	719	888	156	0	0	2,963
1927.....	Illinois No. 1.....	(Dusted.....	150	478	372	1,219	540	27	0	4,538
		(Rusted.....	150	498	437	1,208	268	6	0	3,681

In 1921 all the heads of both rusted and check plants of Mediterranean wheat were studied. As shown in Table 2, the 74 heads of the check plants had 11 spikelets each containing three kernels, whereas in the 65 heads from the rusted plants there was no spikelet that produced three kernels. The heads from the checks averaged 4.7 spikelets and the heads of the rusted plants 1.98 spikelets producing two kernels; the former had an average of 5.9 and the latter plants 4.9 spikelets producing one kernel. The heads of the check plants had an average of 8.3 spikelets and those of the rusted 11.1 spikelets producing no kernels. There was a pronounced increase, therefore, in the number of sterile spikelets in the rusted plants. These were located mostly in the upper and lower portions of the heads. The spikelets of the check plants containing three kernels were located in the central part of the head. The majority of spikelets of the rusted plants containing two kernels were found in the central part of the heads, while in the check plants those containing two kernels were more generally distributed.

In 1922, 47 heads from the check plants and 47 from the late-inoculated plants of Fulcaster were similarly studied. The results (Table 2) agree with those of 1921. The contrast in the numbers of spikelets producing four kernels is especially noticeable, 101 in the checks as compared with 16 in the rusted plants. An additional study of the effect of rust on the development of kernels in different parts of the head was made on a portion of this material.

The location of the kernels in the heads of Fulcaster wheat is illustrated in Table 3 and Figure 3. The numbers of kernels per spikelet are given as they were arranged in 10 heads each from the rusted

and from the check plants. Spikelets producing four kernels are common in the central part of the heads from check plants, while none developed in the rusted ones. Spikelets producing three kernels were found in the upper and lower portions of the heads of the check plants but were restricted largely to the central portion of the heads of the rusted. A much larger number of sterile spikelets occurred in



FIGURE 3.—Effect of leaf rust on the number of kernels per spikelet of Fulcaster wheat in relation to their position on the head: A, Head from check plant (C); B, kernels of each spikelet from A arranged as they occurred in the spikelet; C, kernels of each spikelet from D arranged as they occurred in the spikelet; D, head from a rusted plant (R). The plants from which C was selected were rusted 100 per cent while the heads were still in the boot

the upper and lower portions of the heads of the rusted than in those of the check plants.

In 1927 similar studies were made of heads from rusted and check plants of Michigan Amber wheat in the greenhouse. Michigan Amber usually does not produce so many kernels per spikelet as Fulcaster. The first series studied consisted of heads from pots in which two of the four plants were inoculated and rusted and two were not inoculated and nearly rust free. In the rusted plants there was a

decided reduction in the number of spikelets producing three kernels, as is shown in Table 2 (1927, A). Here, again, the spikelets producing three kernels were more generally distributed throughout the heads of the check plants and more narrowly limited to the central portion of the heads of the rusted plants. The sterile spikelets were somewhat more numerous in the upper and lower portions of the heads of the rusted plants, but the difference was not so noticeable as in the case of the earlier inoculated Fulcaster.

TABLE 3.—*The number of kernels per spikelet in relation to position in heads of rusted and check plants of Fulcaster wheat grown in the greenhouse at La Fayette, Ind., in 1922*^a

Arrangement of spikelets ^b	Heads from rusted plants											Heads from check plants										
	Number of kernels per spikelet in head—										Total kernels	Number of kernels per spikelet in head—										Total kernels
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	
M'	0	0	—	—	—	—	—	—	—	—	0	0	—	—	—	—	—	1	—	—	—	1
L'	0	0	—	—	—	—	—	—	—	—	0	0	0	—	—	—	—	1	2	—	—	2
K'	0	0	0	0	0	—	—	—	—	—	0	0	0	0	—	—	—	—	—	—	—	2
J'	0	0	0	0	0	0	—	—	—	—	0	0	0	0	0	—	—	1	2	—	—	4
I'	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	—	—	2	2	1	1	8
H'	1	0	0	0	0	0	0	0	0	0	0	1	1	0	2	0	2	2	1	1	1	11
G'	1	1	1	0	0	0	0	0	0	0	0	3	2	3	2	3	2	2	2	2	2	22
F'	1	1	2	0	0	0	0	0	0	0	0	4	2	2	2	3	2	2	2	2	2	21
E'	2	2	2	1	0	0	0	0	0	0	0	9	3	3	3	3	3	1	2	3	3	24
D'	2	2	2	2	2	2	2	0	0	0	0	13	3	3	3	3	3	3	2	2	2	27
C'	2	2	2	2	2	2	2	0	0	0	0	15	3	4	3	4	3	3	3	3	3	29
B'	2	2	3	3	2	2	2	0	1	1	0	16	3	4	3	4	3	4	3	3	3	32
A'	3	2	2	2	2	2	2	2	1	1	0	18	4	4	4	4	3	4	4	3	3	34
A	3	3	3	3	3	3	3	1	1	2	0	19	4	4	4	4	4	4	4	3	3	32
B	3	3	3	3	3	3	3	1	1	1	0	21	4	4	4	4	4	4	4	3	3	34
C	3	3	3	3	3	3	3	2	1	3	0	22	4	4	4	4	4	4	4	3	3	35
D	3	3	3	3	3	3	3	2	0	1	0	19	4	4	4	4	4	4	4	3	3	33
E	3	3	2	2	2	2	2	0	0	1	0	12	4	4	4	4	4	3	3	0	0	28
F	2	2	0	0	0	0	0	0	0	0	0	4	4	4	4	4	2	1	4	1	0	25
G	3	2	0	0	0	0	0	0	0	0	0	4	4	3	3	3	3	1	4	1	0	25
H	2	2	0	0	0	0	0	0	0	0	0	4	3	3	3	3	1	2	3	0	0	18
I	2	2	0	0	0	0	0	0	0	0	0	4	3	3	0	0	0	3	0	0	0	9
J	1	0	0	0	0	0	0	0	—	0	0	1	1	0	0	0	0	0	0	0	0	1
K	0	0	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	0
L	0	0	—	—	—	—	—	—	—	—	0	0	0	—	—	—	—	—	—	—	—	0
M	0	0	—	—	—	—	—	—	—	—	0	0	—	—	—	—	—	—	—	—	—	0
Total	—	—	—	—	—	—	—	—	—	—	191	—	—	—	—	—	—	—	—	—	—	453

Summary:		Summary:	
Number of spikelets from rusted plants producing-----	{ 4 kernels=0 3 kernels=24 2 kernels=48 1 kernel=23 0 kernel=124	Number of spikelets from check plants producing-----	{ 4 kernels=42 3 kernels=63 2 kernels=36 1 kernel=24 0 kernel=66

^a As shown in Table 2, 47 heads of each were studied; 10 were selected to illustrate the results obtained.

^b Sequence of letters indicates approximate order of blossoming of spikelets from the central part of the head each way.

The results of a similar study of the heads of Michigan Amber grown in the greenhouse in rows are shown in Tables 2 and 4 and in Figure 4. The heads so produced were much shorter than when the plants were spaced in pots. As shown in Table 2 (1927, B), the rusted plants did not produce any spikelets having four kernels, while 22 such spikelets were produced by the check plants. In the rusted plants there also was a decided reduction in the number of spikelets producing three kernels. Table 4 shows the number of

kernels per spikelet in 13 heads from rusted and 13 from check plants. Here, again, the spikelets producing three and four kernels are abundant in the central portion of the heads of the check, while they are relatively infrequent in the rusted plants. Sterile spikelets are some-

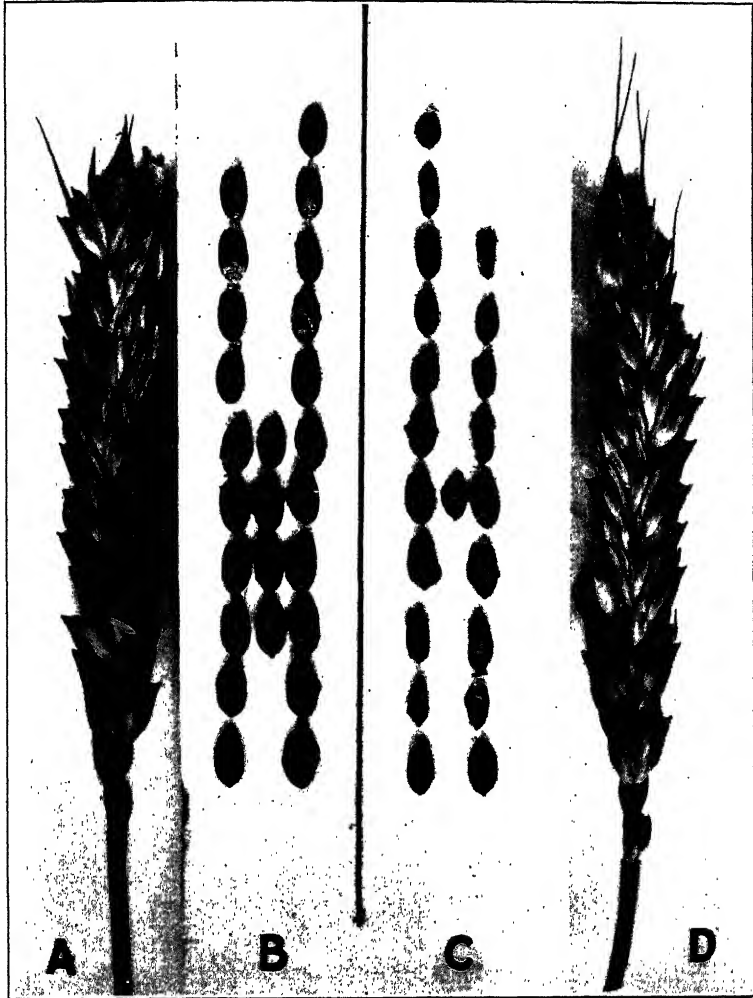


FIGURE 4.—Effect of leaf rust on the number of kernels per spikelet of Michigan Amber wheat in relation to their position on the head: A, Head from check plant (C); B, kernels of each spikelet from A arranged as they occurred in the spikelet; C, kernels of each spikelet from D arranged as they occurred in the spikelet; D, head from a rusted plant (R)

what more abundant in the upper and lower portions of the heads of the rusted plants.

In a similar way, the 150 heads from the dusted and the rusted (undusted) rows of Illinois No. 1 grown in the field in 1927 were studied. The results are given in Tables 2 and 5. As shown in Table 2, there were fewer spikelets producing three and four kernels in the rusted plants. In Table 5 and Figure 5 the kernels per spikelet are

shown as they occurred in 13 heads each of rusted and dusted plants. In both cases the spikelets containing three kernels were located in the central portion of the heads. About twice as many developed in the dusted as in the rusted plants. There was very little difference

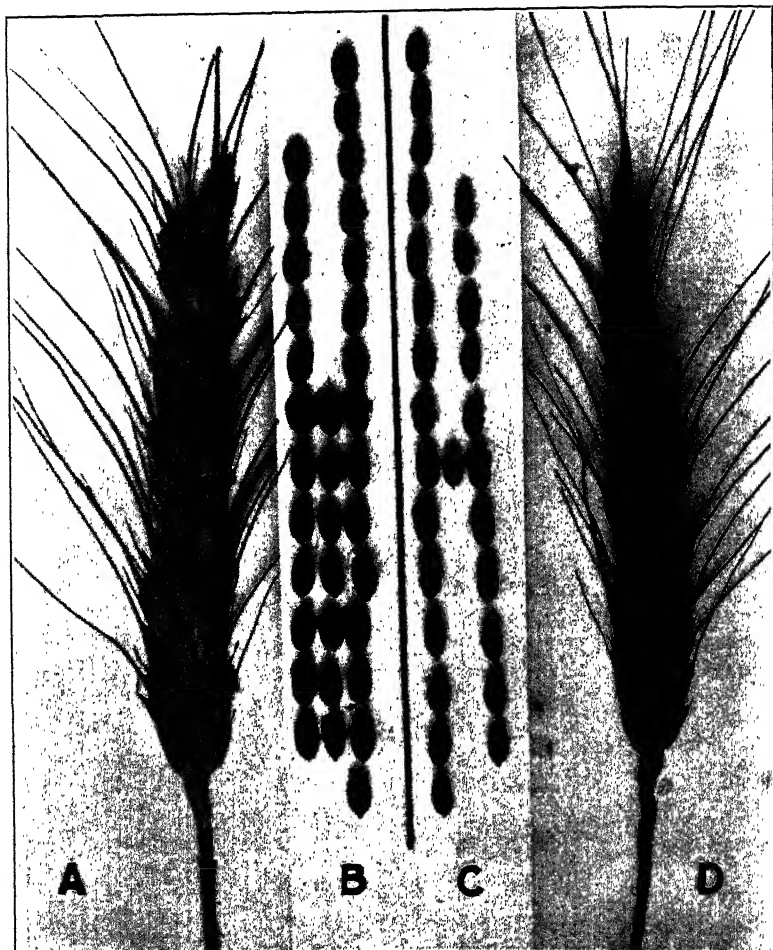


FIGURE 5.—Effect of leaf rust on the number of kernels per spikelet of Illinois No. 1 wheat in relation to their position on the head: A, Head from a plant in the sulphur-dusted plot (D); B, kernels of each spikelet from A arranged as they occurred in the spikelet; C, kernels of each spikelet from D arranged as they occurred in the spikelet; D, head from a heavily rusted plant (R)

in the number of sterile spikelets. The sample of the 13 heads reported in Table 5 would indicate a slight increase in number for the dusted heads. However, as shown in Table 2, the 150 heads from rusted plants had a few more sterile spikelets than the check heads.

TABLE 4.—Number of kernels per spikelet in relation to position in heads of rusted and check plants of Michigan Amber wheat grown in rows in the greenhouse at La Fayette, Ind., in 1927^a

Arrangement of spikelets ^b	Heads from rusted plants													Total kernels	Heads from check plants													Total kernels
	Number of kernels per spikelet in head—														Number of kernels per spikelet in head—													
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13	
H'-----	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	2	0	0	0	1	1	1	1	1	9	
G'-----	1	2	0	1	1	1	0	0	0	0	0	1	0	1	7	2	0	1	2	0	2	2	1	1	1	1	17	
F'-----	1	1	1	1	2	2	1	1	1	0	1	1	0	1	13	2	1	1	2	1	2	2	2	2	1	2	21	
E'-----	1	2	1	2	1	2	2	2	1	2	1	1	1	2	20	2	1	1	2	1	3	1	2	2	2	1	22	
D'-----	2	2	1	2	2	2	2	2	0	2	0	2	2	2	21	2	2	2	2	2	2	2	2	2	2	1	26	
C'-----	2	2	1	1	2	2	2	2	1	2	1	2	1	2	20	2	2	2	2	2	3	3	3	3	2	2	29	
B'-----	2	3	2	2	2	2	2	2	1	2	2	2	3	1	26	2	2	1	2	3	3	3	3	2	2	2	29	
A'-----	2	2	0	2	3	1	1	2	2	2	2	2	3	1	23	2	1	1	2	1	3	3	3	3	2	3	27	
A-----	1	2	2	2	2	2	1	2	2	2	2	2	3	1	24	2	1	2	1	2	4	3	3	3	2	2	29	
B-----	1	2	0	2	3	2	0	2	1	2	2	2	3	1	20	2	1	2	1	2	4	3	3	3	2	1	27	
C-----	0	2	0	2	3	2	0	1	2	2	2	1	2	0	17	2	0	1	0	1	4	3	3	3	2	2	23	
D-----	0	0	0	0	1	1	2	0	0	2	2	2	2	3	12	1	0	0	0	0	4	2	3	3	1	0	16	
E-----	0	0	0	0	0	0	0	0	0	2	2	2	2	3	9	0	0	0	0	0	2	0	0	0	0	0	6	
F-----	0	0	0	0	0	0	0	0	0	2	0	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	3	
G-----	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	
H-----	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	
I-----	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total-----															216												287	

Summary:		Summary:	
Number of spikelets from rusted plants producing-----	4 kernels= 0 3 kernels=10 2 kernels=71 1 kernel =44 0 kernel =96	Number of spikelets from check plants producing-----	4 kernels= 4 3 kernels=27 2 kernels=76 1 kernel =38 0 kernel =76

^a As shown in Table 2, 246 heads of rusted plants and 241 heads of the check plants were studied; 13 heads were selected to illustrate the results obtained.

^b Sequence of letters indicates approximate order of blossoming of spikelets from the central part of the head each way.

This distribution of kernels is significant when the sequence of blossoming is taken into consideration. Blossoming usually starts in the central portion of the head. The outer flowers of the spikelets blossom first, followed by the inner flowers in their order on the rachilla. Blossoming progresses upward and downward from the central portion of the head. As the upper and lower spikelets, and the inner flowers of the central spikelets, more often fail to develop kernels in the heads of rusted plants than in those of the dusted plants, it is evident that infection prevents the development of kernels in the late-blooming flowers.

TABLE 5.—Number of kernels per spikelet in relation to position in heads of rusted and sulphur-dusted plants of Illinois No. 1 wheat grown in the field nursery at La Fayette, Ind., 1927^a

Arrangement of spikelets ^b	Heads from rusted plants													Heads from check (dusted) plants												
	Number of kernels per spikelet in head—												Total kernels	Number of kernels per spikelet in head—												Total kernels
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13
L'																										
K'																										
J'	1													2	1											
I'	1													3	1											
H'	1													8	2	2	1	1	1	0						
G'	1	0												16	2	2	2	2	2	2	1	1	1	1	1	1
F'	2	2												20	2	2	2	2	2	2	2	2	2	2	2	2
E'	2	2												20	2	2	2	2	2	2	2	2	2	2	2	2
D'	2	2												26	2	2	2	2	2	2	2	2	2	2	2	2
C'	2	2												26	2	2	2	2	2	2	2	2	2	2	2	2
B'	2	2												26	2	2	2	2	2	2	2	2	2	2	2	2
A'	2	2												25	2	2	2	2	2	2	2	2	2	2	2	2
A	2	2												29	3	3	3	3	3	3	3	3	3	3	3	3
B	2	2												28	3	3	3	3	3	3	3	3	3	3	3	3
C	2	2												28	3	3	3	3	3	3	3	3	3	3	3	3
D	2	2												29	3	3	3	3	3	3	3	3	3	3	3	3
E	2	2												26	3	3	3	3	3	3	3	3	3	3	3	3
F	3	1												23	3	3	3	3	3	3	3	3	3	3	3	3
G	1	0												16	3	1	1	1	2	0	2	2	2	2	2	2
H	0	0												7	2	0	0	0	0	0	0	0	0	0	0	0
I	0	0												2	0	0	0	0	0	0	0	0	0	0	0	0
J	0	0												0	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0												0	0	0	0	0	0	0	0	0	0	0	0	0
L	0	0												0	0	0	0	0	0	0	0	0	0	0	0	0
Total													336													428

Summary:

Number of spikelets from rusted plants producing.....

3 kernels= 25
2 kernels=108
1 kernel = 45
0 kernel = 33

Summary:

Number of spikelets from check plants producing.....

3 kernels=65
2 kernels=97
1 kernel = 39
0 kernel = 41

^a As shown in Table 2, 150 heads of each were studied; 13 were selected to illustrate the results obtained.

^b Sequence of letters indicates approximate order of blossoming of spikelets from the central part of the head each way.

EFFECT ON OVERWINTERING OF WHEAT

In the fall of 1922 a series of field sowings was made to obtain information on the effect of the development of leaf rust on wheat sown in the fall. Seed of the Trumbull variety was sown on three different dates, September 8, September 20, and October 3, in plots 17 by 50 feet. In each of these plots six areas, each 3 feet square, were covered with muslin cages. (Fig. 6, A.) Plants in three of these cages were inoculated with the leaf rust of wheat, and those in three others, alternating with the first three, were maintained free from rust.

In the first plot, sown September 8, inoculations were made on September 20. By October 4 a moderate infection had developed on the older leaves. The rust increased slowly during October, the new leaves becoming infected as they developed; a severe infection had developed by November 13. No rust developed in the check cages, and the wheat from them will be designated hereafter as rust free.

In the second plot, sown September 20, plants in three of the cages were inoculated on October 15. A slight amount of rust had appeared by October 31, and by November 13 a moderate infection had developed on the older leaves. No rust developed in the cages containing the check plants.

The third plot, sown October 3, was inoculated on October 25, and a slight scattered infection had developed by November 13. No rust occurred in the check cages. On November 21 all the cages were removed. Both the infected and rust-free wheat under the cages had

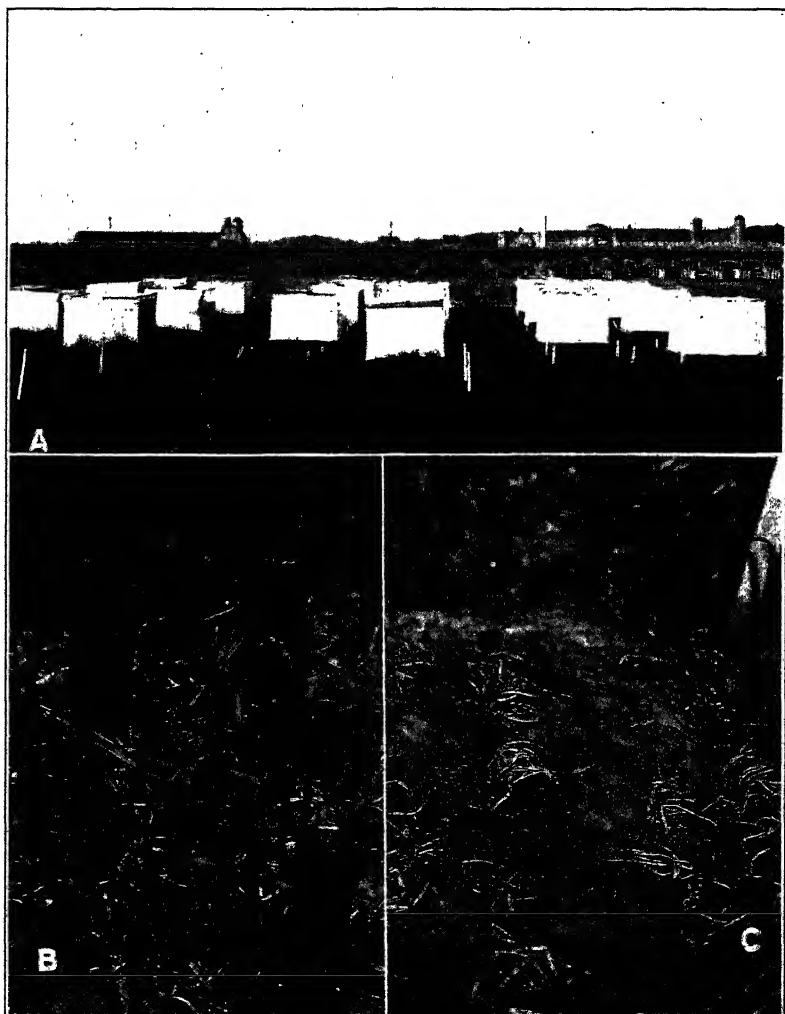


FIGURE 6.—Effect of leaf rust on winterkilling of wheat: A, Early fall sowing showing caged areas; B, spring survival in an area free from rust in the fall; C, spring survival in one of the areas severely rusted in the fall

made rank growth and therefore was not in good condition for overwintering.

On May 2, counts were made to determine the survival. The average stand in rusted areas of the first sowing was 4.1 per cent. (Fig. 6, C.) The average for the rust-free areas was 77.7 per cent. (Fig. 6, B.) In the second sowing the stand in the rusted areas was 17.6 per cent and in the rust free 77.7 per cent. In the third sowing the rusted areas had a stand of 48.6 per cent and the rust free 55.1 per cent.

In the case of the early sown and severely rusted wheat, the infection was responsible for a very pronounced increase in winterkilling. The second sowing was less severely rusted and the rusted areas showed less winterkilling, although almost four times as much as the check. The inoculated wheat of the late sowing was only slightly infected, and there was no very significant difference between the winterkilling of these rusted areas and the rust-free check areas. However, in the late sowing, there was a considerable increase of winterkilling in the rust-free plots over similar plots of the earlier sowings. Unquestionably, the effect produced by caging was responsible for a portion of the winterkilling. However, the results indicate that leaf rust, when present in abundance, may be a considerable factor in causing winterkilling.

DISCUSSION

The results of these investigations indicate that the effect of the leaf rust of wheat varies with the severity of infection and the stage of development of the wheat. Under the conditions of these experiments, a moderate infection occurring from early in the wheat development (tillering) to maturity resulted in 57.2 to 63.3 per cent reduction in yield. A severe infection occurring throughout the same period was responsible for a reduction of 97.4 per cent. A somewhat later severe infection reduced the yield 91.3 per cent. A severe infection developing just before the heads emerged from the boot resulted in a reduction of 54.3 per cent. Severe infections occurring from head emergence to maturity resulted in 37.2 per cent reduction. Severe infection from blossoming to maturity produced 24 to 33 per cent reduction. Moderate infection, following blossoming and increasing to a maximum before maturity, resulted in 11.1 per cent reduction.

Reduced yield results from production of fewer and also of lighter kernels by the rusted plants. In the case of infections occurring relatively early, a large proportion of the reduction in yield was due to fewer kernels, although the reduction in weight of the kernels is an important factor. In the case of late infections, the reduction in number of kernels is still an important factor, but the reduction in weight per kernel plays a proportionately greater part.

Although no critical study of the effect of leaf rust on the quality of the grain was made, there appears to be no very outstanding difference. Under conditions of severe infection over fairly long periods, rusted plants produced more shriveled kernels. Field observations indicate that under conditions of severe drought abundant leaf-rust infection may result in considerable shriveling. According to Weaver (16), H. B. Humphrey found that the leaf rust of wheat had a very pronounced effect upon transpiration, increasing it 38 per cent when the uredinia of the rust occupied less than 1 per cent of the transpiring surface. Under conditions of drought, leaf rust therefore may be of unusual importance.

In one experiment in 1927, leaf rust apparently caused an increase in the amount of yellow berry. As no differences in this respect were noted in other experiments, it is questionable how important the rust may be in that connection. It suggests that under conditions favoring the production of yellow berry the disturbance in metabolism produced by the rust sometimes may increase the proportion of such kernels. Usually, however, it would appear that leaf rust has no

pronounced effect upon the quality of grain, and this probably accounts in part for the earlier opinions that leaf rust was of little or no importance in wheat production.

Leaf rust reduces not only the yield of grain but also that of the straw. The severe infections occurring early prevented the development of many culms and caused a reduction of 70 per cent or more. Later infections reduced the weight of straw from 11 to 33 per cent. While no analyses of straw were attempted, the effect on color is suggestive. The Michigan Amber variety in 1927 developed purple color in the check plants when they were maturing, while the rusted ones were a dull yellow. This would indicate a shortage of carbohydrates in the rusted plants.

That the amount of functioning leaf area is important in determining the yield of wheat has been demonstrated by a number of investigators. Kiesselbach (6) studied the effect of removing leaves at different stages in plant development. When leaves were removed 3 days after heading (25 days before maturity) the yield of grain was reduced from 36.9 to 28.6 gm., or 22.5 per cent. The weight of straw was reduced from 4,284 to 3,603 gm., or 15.8 per cent. When the leaves were removed 10 days after heading (18 days before maturity) the weight of grain was 32.4 gm., a reduction of 12.2 per cent, and the weight of straw was 3,796 gm., a reduction of 11.3 per cent. When the leaves were removed 17 days after heading (11 days before maturity) the yield of grain was 35.4 gm., a reduction of 4.0 per cent, and the yield of straw was 4,050 gm., a reduction of 5.4 per cent. Roebuck and Brown (14) studied the effect on stripped (all the leaves removed), half-stripped (one-half of each leaf removed), and bottom-stripped (all the lower leaves removed) plants for periods of one, two, three, four, five, six, and seven weeks. Expressing their data in terms of percentage reduction in grain produced, the yield of plants of both varieties completely stripped for a period of seven weeks was reduced 42.9 per cent, for four weeks 24.9 per cent, and for one week 5.5 per cent. The yield of plants with half of each leaf removed for seven weeks was reduced 48.9 per cent, for four weeks 19.0 per cent, and for one week 10.6 per cent. The yield of plants with all the lower leaves removed for seven weeks was reduced 41.4 per cent, for four weeks 18.8 per cent, and for one week 4.8 per cent. Leaf rust not only reduces the leaf area of the wheat plant but also modifies the metabolism of its host. According to Weaver (16), it also increases its rate of transpiration. Other functions probably also are seriously affected, for Reed and Cooley (13) have shown that in apple leaves infected with *Gymnosporangium juniperi-virginianae* the average rate of assimilation of carbon dioxide was only about one-half that in the healthy plants.

That severe infection of a highly resistant variety may cause a decided decrease in yield is shown by the results obtained with the Webster variety. As has been stated, physiologic form 5 causes a rapid necrosis resulting in a production of small flecks and the death of the rust. When plenty of inoculum is present and abundant infections occur, the leaves of such infected plants die earlier than do those of check plants. The resulting reduction in functional leaf area therefore should bring about a reduction in yield. The 11.4 per cent reduction in yield noted for Webster is well within the expectation for the reduction of functioning leaf area. In nurseries where

varieties are being compared in rows, resistant varieties usually are subject to severe infection from neighboring susceptible varieties and the yield probably is reduced. However, where such resistant varieties are sown in large areas, especially in the absence of susceptible varieties, such an effect is not to be expected. Infection from distant susceptible varieties will be slight, and if the rust is unable to develop spores on the resistant variety it can not multiply and spread. Tests of resistant varieties in close comparison with susceptible varieties probably do not indicate the full yielding ability of the resistant varieties when sown in large areas.

A study of the effect of the rust upon kernel production in comparison with blossoming indicates that there is a fairly close correlation with time of blossoming. The first flowers to open are the outer ones of the central spikelets. Blossoming then progresses up and down in the spike and inward in the spikelets. In rusted plants the grain is produced only by the earlier flowers—the outer of the central spikelets and a portion of the upper and lower spikelets. The check plants develop grain also in the inner flowers of the central spikelets and in more of the outer flowers of the upper and lower spikelets. With increased severity of the rust the kernel production is more closely restricted to the earliest flowers. This indicates that the rust is responsible for a reduction in the materials necessary for kernel development. Under such conditions the first flowers to be pollinated obtain the available material and develop, while the later flowers are unable to develop because of lack of available material. Even in the early flowers the shortage of material results in somewhat smaller and lighter kernels. The effect of very late infection, after kernel development of the head in general is in a fairly advanced stage, is largely a reduction in weight of kernels, especially in the late flowers.

Severe autumnal infection of winter wheat may be an important factor favoring winterkilling, as has been shown by the marked increase in winterkilling where early sown wheat was heavily rusted. Infection on fall-sown grain takes place from volunteer wheat, and the spread from such sources usually is slow. Over a considerable portion of the soft-winter-wheat belt wheat is sown late, usually about October 1 in the vicinity of La Fayette, Ind., in order to escape infestation with the Hessian fly. Where such is the practice, the natural infection from volunteer wheat usually is rather slight. With earlier sowings the chances for development of the rust increase, and when conditions are favorable for infection and spread leaf rust may be an important factor affecting winter survival.

The leaf rust of wheat is more or less prevalent throughout the wheat-growing areas of the United States. It is especially prevalent and severe in the soft-winter-wheat area. Since 1917 the Plant Disease Survey of the Bureau of Plant Industry, United States Department of Agriculture, has been collecting information from collaborators in various States concerning the prevalence and severity of plant diseases. A review of this information shows that in the soft-winter-wheat area, at least, leaf rust has been present to some extent each year. In 1918, as summarized by Haskell,⁸ leaf rust was particularly

⁸ HASKELL, R. J. Op. cit. (See footnote 4.)

severe in the eastern and southeastern United States. The estimated average percentages of total leaf area affected, as determined by a survey throughout the eastern wheat area, are shown in Table 6. It is interesting to note that for 1918 no collaborator of the Plant Disease Survey⁹ estimated the reduction in yield due to leaf rust as more than a trace.

TABLE 6.—*Estimated average percentages of total leaf area of wheat affected by leaf rust in 1918 in the States named*

[U.=unreported because loss was insignificant; T.=trace]

Section and State	Leaf area affected	Section and State	Leaf area affected
New England:	<i>Per cent</i>	South-Central, East—Continued.	<i>Per cent</i>
Maine.....	15.0	Georgia.....	26.3
New Hampshire.....	12.0	Alabama.....	30.5
Vermont.....	19.0	Mississippi.....	25.9
Massachusetts.....	7.6	Kentucky.....	30.3
Connecticut.....	9.2	Tennessee.....	28.0
North-Central, East:		North-Central, West:	
New York.....	17.0	Minnesota.....	7.0
Pennsylvania.....	48.7	Iowa.....	13.0
Delaware.....	U.	North Dakota.....	U.
New Jersey.....	43.0	South Dakota.....	9.6
Maryland.....	28.9	Nebraska.....	5.0
West Virginia.....	47.9	Colorado.....	T.
Ohio.....	28.8	Montana.....	T.
Indiana.....	U.	Wyoming.....	T.
Michigan.....	20.6	South-Central, West:	
Wisconsin.....	39.7	Missouri.....	8.3
Illinois.....	6.5	Kansas.....	5.2
South-Central, East:		Arkansas.....	U.
Virginia.....	37.9	Oklahoma.....	6.6
North Carolina.....	29.7	Louisiana.....	55.8
South Carolina.....	35.8	Texas.....	1.4

In 1919 the leaf rust of wheat was unusually severe. Johnson and Haskell,¹⁰ in their summary of cereal diseases for that year, stated that some fields in Georgia and Arkansas were so severely infected that they were abandoned in the spring of 1919. In the southeastern United States the winter was mild and the rust developed late into the winter and resumed growth early in the spring, resulting in early severe infections. Northward the rust was less severe, although wheat was heavily rusted. In the Western and Northwestern States the rust was relatively slight except locally in certain irrigated sections. Estimates of the leaf surface infected are not given. However, the rust was of such severity that a definite loss was estimated for the various States in the eastern half of the country varying from 0.5 to 8 per cent reduction in yield. The estimated reduction in yield due to leaf rust, as summarized by the Plant Disease Survey,¹¹ is shown in Table 7. On this basis the loss from leaf rust for the United States in 1919 was 1.47 per cent, or 16,633,000 bushels.

⁹ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES—1918. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 6: 186-213. 1919. [Mimeographed.]

¹⁰ JOHNSON, A. G., and HASKELL, R. J. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1919. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 8: 1-81, illus. 1920. [Mimeographed.]

¹¹ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1919. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 12: 307-332. 1920. [Mimeographed.]

TABLE 7.—Estimated percentage annual and 9-year average reduction in wheat yield in the 9 years from 1919 to 1927, inclusive, in the States named

[U.=unreported because loss was insignificant; T.=trace]

Section and State	Estimated percentage reduction in yield									9-year average
	1919	1920	1921	1922	1923	1924	1925	1926	1927	
New England:										
Maine.....	U.	U.	U.	U.	U.	U.	U.	U.	U.	U.
New Hampshire.....	U.	U.	U.	U.	U.	U.	U.	U.	U.	U.
Vermont.....	0.5	T.	0	U.	1	0.5	U.	U.	U.	0.2
Massachusetts.....	U.	U.	0	T.	U.	U.	U.	U.	U.	0
Connecticut.....	.5	U.	U.	T.	U.	U.	0.25	U.	U.	.08
Rhode Island.....	U.	U.	U.	U.	U.	U.	U.	U.	U.	U.
North-Central, East:										
New York.....	1	T.	3	3	1	1.5	1	1	1	1.4
Pennsylvania.....	1	T.	2	4	1.5	1	.3	.5	6	1.8
Delaware.....	2	.2	3	2	.2	.1	T.	T.	T.	.8
New Jersey.....	1	T.	10	U.	.2	T.	T.	.5	U.	1.3
Maryland.....	2	1.5	6	5	1.5	2	1	.5	.5	2.2
West Virginia.....	3	T.	5	18	1	T.	T.	T.	1	3
Ohio.....	1	T.	4	5	4	T.	.1	T.	.5	1.6
Indiana.....	1	.1	7	10	2	T.	T.	.5	13	4.2
Michigan.....	1	T.	U.	2	T.	.5	0	2	1	.7
Wisconsin.....	1	T.	T.	1	T.	1	1	1	T.	.6
Illinois.....	1	.5	1	10	3	2.4	1.5	1	2.5	2.5
South-Central, East:										
Virginia.....	3	2	5	5	1	3	T.	T.	1.5	2.1
North Carolina.....	5	3	20	U.	3	1	U.	T.	3	4.7
South Carolina.....	5	2	2	10	2	2	1	1	2	3.0
Georgia.....	8	2	3	5	5	5	2	U.	U.	3.4
Alabama.....	5	2	U.	3	5	U.	U.	U.	U.	1.7
Mississippi.....	5	4	3	5	1	0	U.	U.	U.	1.7
Kentucky.....	2	1	U.	10	1	T.	.1	T.	U.	1.6
Tennessee.....	4	T.	10	15	2	U.	1	T.	20	5.8
North-Central, West:										
Minnesota.....	1	T.	T.	.5	T.	T.	T.	T.	3	.5
Iowa.....	1.5	1	T.	T.	1.5	T.	.5	6	15	3.3
North Dakota.....	1	1.5	3	1	1	T.	T.	T.	2	1.1
South Dakota.....	.5	1	5	T.	1	0	0	T.	3	1.2
Nebraska.....	1.5	1	U.	U.	1	T.	T.	T.	2	.6
Montana.....	0	0	1	T.	U.	T.	0	0	T.	.1
Wyoming.....	U.	U.	U.	T.	U.	0	0	U.	U.	0
Colorado.....	.1	T.	U.	T.	1	0	0	0	T.	.1
South-Central, West:										
Missouri.....	2	1	U.	U.	1	U.	U.	0	T.	.4
Kansas.....	1.5	T.	1	1	1	T.	0	T.	8	1.4
Arkansas.....	8	2	5	5	4	T.	T.	T.	U.	2.7
Oklahoma.....	4	2	5	5	U.	0	U.	U.	U.	1.8
Louisiana.....	U.	T.	U.	U.	U.	T.	T.	U.	U.	0
Texas.....	4	1	T.	1	1	1	.5	1	3	1.4
New Mexico.....	U.	T.	U.	U.	U.	U.	U.	U.	T.	0
West:										
Idaho.....	0	T.	0.5	0	T.	0	T.	T.	T.	.06
Utah.....	U.	T.	.2	T.	.3	.1	.5	U.	U.	.1
Arizona.....	U.	T.	U.	U.	U.	U.	U.	.5	T.	.06
Washington.....	0	T.	T.	0	T.	T.	0	T.	T.	0
Oregon.....	0	T.	T.	0	T.	T.	T.	.5	T.	.06
California.....	T.	T.	2	T.	T.	0	1	U.	U.	.3
Nevada.....	T.	T.	U.	0	U.	U.	U.	U.	U.	0

Fromme,¹² in his summary of cereal diseases for 1920 for the Plant Disease Survey, stated that leaf rust in general was less severe than in 1919. It was most prevalent and destructive in the Southern States, less prevalent in a belt from Pennsylvania westward to Kansas and Iowa, and more prevalent in North Dakota, Minnesota, and upper Michigan. The estimate of losses in yield for 1920 as given by the Plant Disease Survey¹³ is shown in Table 7. The estimated total reduction for the United States is given as 0.6 per cent, or 5,318,000 bushels.

¹² FROMME, F. D. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1920. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 15: 115-176, illus. 1921. [Mimeographed.]

¹³ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1920. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 18: 317-338. 1921. [Mimeographed.]

Stakman,¹⁴ in his summary of cereal diseases for 1921 for the Plant Disease Survey, reported that leaf rust probably was more prevalent than it had been for several years. The rust was abundant on wheat in the fall of 1920, and in the South it developed to some extent throughout the winter. Various observers noted that it developed early in the spring. This resulted in an early, severe infection in which not only the leaves but in some cases the glumes also were severely rusted, resulting in premature ripening. In West Virginia and Oklahoma drought was considered to have increased the resultant damage. The rust was much more prevalent on the Pacific coast than in the previous two years, causing damage especially in the valleys. As pointed out by Stakman, the rust was most prevalent and severe in the soft-winter-wheat area. The estimated reduction in yield for 1921 as given by the Plant Disease Survey¹⁵ is shown in Table 7. The estimated reduction for the United States is given as 1.9 per cent, or 16,944,000 bushels.

Haskell and Wood,¹⁶ in their summary of cereal diseases for 1922 for the Plant Disease Survey, show that the leaf rust of wheat was again very severe throughout the soft-winter-wheat area. It apparently was more generally destructive than in any previous year. The rust was rated as the most important disease of wheat in 1922 by those reporting from New York, Delaware, Maryland, Virginia, West Virginia, South Carolina, Indiana, Illinois, Tennessee, Mississippi, Oklahoma, and Arkansas, and as the disease second in importance by those in Pennsylvania, Georgia, Ohio, Wisconsin, and North Dakota. In Georgia the rust was severe early in the plant development, when the wheat was about 1 foot high. The leaves were prematurely killed, and farmers were so alarmed that they inquired concerning the advisability of cutting their wheat for hay, pasturing it, or plowing it under. At Experiment, Ga., Prof. R. P. Bledsoe estimated the loss as likely to reach 50 per cent, stating that wheat was yielding 14 bushels there as compared with 30 bushels in the year before. In Virginia, Tennessee, and Kentucky, leaf rust started somewhat later in the spring but reached a severity comparable with that of the previous season. In southern Indiana it was one of the principal factors contributing toward low yields, it being estimated as bringing about a 20 per cent reduction. Tehon (15) placed the destruction of leaf area in Illinois at 50.3 per cent.

The Plant Disease Survey¹⁷ gave the estimates of reduction in yield for 1922, as shown in Table 7. The reduction in yield for the entire United States for 1922 is given as 2.5 per cent, or 23,107,000 bushels.

Leaf rust was less destructive in 1923 than in the two previous seasons.¹⁸ The winter of 1922-23 was unfavorable for the overwintering of the rust, and the cool spring and early summer held it in check. Consequently, the rust was slow in developing and reached a maxi-

¹⁴ STAKMAN, E. C. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1921. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 21: 139-254, illus. [Mimeographed.]

¹⁵ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1921. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 24: 489-510. 1922. [Mimeographed.]

¹⁶ HASKELL, R. J., and WOOD, J. I. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Bul. 27: 164-266, illus. 1923. [Mimeographed.]

¹⁷ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1922. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 30: 462-490. 1923. [Mimeographed.]

¹⁸ HASKELL, R. J. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1923. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 35: 244-317, illus. 1924. [Mimeographed.]

mum only a short time before harvest. The reduction in yield for 1923, as given by the Plant Disease Survey,¹⁹ is shown in Table 7. The reduction in yield in 1923 for the entire United States is given as 1.09 per cent, or 9,735,000 bushels.

In general there was much less leaf rust in 1924 than in 1923.²⁰ Many observers commented on the slight development. In Georgia it was very late, reaching only a moderate infection before harvest. In Tennessee it was the least that had been noted for a number of years. In Indiana it was considered the least in 6 years and in Kansas the least in 10 years. Tehon (15) gave the leaf area infected in Illinois as 19.1 per cent, compared with 31.3 per cent in 1923 and 50.3 per cent in 1922. Various factors contributed to this condition. An open winter with alternate freezing and thawing, and consequent destruction of the infected leaves of winter wheat, greatly reduced the overwintering of the rust. The generally cool spring retarded its development. The estimated reduction in yield in 1924 for the United States, according to the Plant Disease Survey,²¹ is shown in Table 7. The total estimated reduction for the United States is given as 0.23 per cent, or 2,213,000 bushels.

In 1925 leaf rust was less severe than in 1924.²² The overwintering of the rust was reduced in many places by low temperatures and insufficient protection. The months of April, May, and June were unusually dry in the eastern part of the country, and rust development was greatly retarded. Tehon (15) gave the leaf area infected in Illinois as 17.1 per cent, compared with 19.1 per cent in 1924. In New York, Kightlinger and Whetzel (8) reported increasing the yield of winter wheat 18.5 per cent by reducing leaf-rust infection 48.2 per cent by dusting with sulphur. The reduction in yield for 1925, as estimated by the Plant Disease Survey,²³ is shown in Table 7. The total reduction for the United States is given as 0.2 per cent, or 1,814,000 bushels.

The leaf rust of wheat was slightly more prevalent and destructive in 1926 than in 1925.²⁴ It by no means approached its prevalence during the period from 1918 to 1923, inclusive. In the southeastern United States an unusually dry spring prevented rust development to such an extent that only a trace occurred throughout much of the region, moderate infection developing very late locally, especially in low areas. Northern Texas and southern Oklahoma had heavy infections. Northern Oklahoma and southern Kansas had a moderate amount of rust early in the season, but dry weather prevented much further increase. There was a slight overwintering of leaf rust in Indiana, but development was retarded by cool weather. A moderate to heavy infection developed late. A similar situation occurred in

¹⁹ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1923. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 36: 318-348. 1924. [Mimeographed.]

²⁰ MELCHERS, L. E. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1924. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 40: 106-191, illus. 1925. [Mimeographed.]

²¹ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1924. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 43: 381-410. 1925. [Mimeographed.]

²² HASKELL, R. J. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1925. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 48: 301-381, illus. 1926. [Mimeographed.]

²³ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1925. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 49: 382-412. 1926. [Mimeographed.]

²⁴ KIRBY, R. S., and ARCHER, W. A. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1926. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 53: 110-208, illus. 1927. [Mimeographed.]

Wisconsin. The reduction in yield for 1926 for the United States, as estimated by the Plant Disease Survey,²⁵ is shown in Table 7. The total reduction for the United States is given as 0.4 per cent, or 3,990,000 bushels.

Leaf rust was considerably more prevalent and destructive in 1927²⁶ than in previous years. There apparently was considerable overwintering, especially in the southern part of the country. From Pennsylvania was reported one of the most severe epidemics ever occurring in that State, with an average infection of 62 per cent. Low temperatures apparently held the rust somewhat in check throughout Indiana and Missouri, but a maximum development was reached before maturity. As shown in this paper, 11.1 per cent reduction in yield of winter wheat occurred in plots at La Fayette, Ind. The loss for the State probably averaged about 13 per cent. From Wisconsin was reported heavy leaf-rust infection in the milk stage of wheat development. The overwintering of rust throughout Texas, Oklahoma, and Kansas resulted in heavy infections early in the season. Reductions in yield of 2 to 20 per cent were reported from Kansas. In Iowa infections were very severe. ("Some fields were so severely infected early in the season that heads never formed, in others the plants were so badly checked in their growth that heads did not fill properly."—Archer.)²⁷ Infections of 80 to 100 per cent were common.

In the spring-wheat area, leaf rust was more prevalent and destructive than at any time during the period from 1918 to 1926, inclusive. It was reported as very severe on spring wheat in North Dakota. Plants were practically defoliated and heads ripened early in Minnesota. Stakman, according to Haskell,²⁸ estimated reductions in yield of susceptible varieties in Minnesota as 5 to 7 bushels per acre, based on results of sulphur-dusting experiments. The estimated reductions in yield for 1927²⁹ are given in Table 7. The reduction in yield for the United States in 1927, due to leaf rust, is given as 29 per cent, or 28,626,000 bushels.

The States, arranged in descending order of the average reduction in yield due to leaf rust in the period from 1919 to 1927, inclusive, are: Tennessee, 5.8 per cent; North Carolina, 4.7 per cent; Indiana, 4.2 per cent; Georgia, 3.4 per cent; Iowa, 3.3 per cent; West Virginia, 3.1 per cent; South Carolina, 3.0 per cent; Arkansas, 2.7 per cent; Illinois, 2.5 per cent; Maryland, 2.2 per cent; Virginia, 2.1 per cent; Oklahoma, 1.8 per cent; Pennsylvania, 1.8 per cent; Alabama, 1.7 per cent; Mississippi, 1.7 per cent; Kentucky, 1.6 per cent; Ohio, 1.6 per cent; New York, 1.4 per cent; Kansas, 1.4 per cent; Texas, 1.4 per cent; New Jersey, 1.3 per cent; South Dakota, 1.2 per cent; North Dakota, 1.1 per cent; Delaware, 0.8 per cent; Michigan, 0.7 per cent; Wisconsin, 0.6 per cent; Nebraska, 0.6 per cent; Minnesota, 0.5 per cent; Missouri, 0.4 per cent; California, 0.3 per cent; Vermont, 0.2 per cent; Colorado, 0.1 per cent; Montana, 0.1 per cent; Utah, 0.1 per cent; Connecticut, 0.08 per cent; Idaho, 0.06 per cent.

²⁵ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1926. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 56:394-423. 1927. [Mimeographed.]

²⁶ HASKELL, R. J. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1927. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 62:302-353. 1928. [Mimeographed.]

²⁷ HASKELL, R. J. Op. cit. (p. 314).

²⁸ HASKELL, R. J. Op. cit. (See footnote 22.)

²⁹ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1927. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 64:370-399. 1928. [Mimeographed.]

cent; Arizona, 0.06 per cent; Oregon, 0.06 per cent; and Louisiana, Maine, Massachusetts, Nevada, New Hampshire, New Mexico, Rhode Island, Washington, and Wyoming, no loss large enough to be estimated. This shows that the rust was most severe in the soft-winter-wheat area, especially in the eastern and southeastern portions.

The losses due to the rust in some of the hard-winter-wheat and spring-wheat States are much greater than the percentage reduction would indicate, due to large acreages in such States as Kansas, Oklahoma, North Dakota, and South Dakota, as compared with the small acreages of such Southern States as Georgia, South Carolina, Alabama, and Mississippi, where the rust is much more severe. The total estimated reduction in yield in bushels for the 9-year period from 1919 to 1927, inclusive, by States, is shown in Table 8. The estimated total loss for the United States in the nine years is 108,380,000 bushels, or an average loss of 12,042,000 bushels per year.

Judging from the results obtained in these studies, the estimates of losses as determined by the collaborators for the Plant Disease Survey have been conservative. The leaf rust of wheat is more or less prevalent each year from the Rocky Mountains eastward. It is most prevalent and severe in the soft-winter-wheat States, and virtually every year it is a factor of importance in the production of soft winter wheat. While in both the hard-winter-wheat and spring-wheat States the rust is not so prevalent, the loss is important on account of the large acreage involved. When the rust is severe, as in 1926 in the hard-winter-wheat and in 1927 in the spring-wheat areas, it is a very important factor in wheat production in these areas.

TABLE 8.—*Estimated total reduction in wheat production, by States, caused by leaf rust, in the 9-year period from 1919 to 1927, inclusive*

[U.=unreported because the loss was insignificant]

Section and State	Reduction in produc- tion (bushels)	Section and State	Reduction in produc- tion (bushels)
New England:		North-Central, West:	
Maine.....	U.	Minnesota.....	1,660,000
New Hampshire.....	U.	Iowa.....	3,345,000
Vermont.....	2,000	North Dakota.....	10,280,000
Massachusetts.....	U.	South Dakota.....	4,213,000
Connecticut.....	U.	Nebraska.....	3,717,000
Rhode Island.....	U.	Montana.....	316,000
North-Central, East:		Wyoming.....	U.
New York.....	1,057,000	Colorado.....	221,000
Pennsylvania.....	4,388,000	South-Central, West:	
Delaware.....	125,000	Missouri.....	2,087,000
New Jersey.....	208,000	Kansas.....	16,413,000
Maryland.....	2,119,000	Arkansas.....	472,000
West Virginia.....	1,155,000	Oklahoma.....	7,726,000
Ohio.....	6,882,000	Louisiana.....	U.
Indiana.....	13,207,000	Texas.....	3,046,000
Michigan.....	1,261,000	New Mexico.....	U.
Wisconsin.....	208,000	West:	
Illinois.....	13,497,000	Idaho.....	147,000
South-Central, East:		Utah.....	127,000
Virginia.....	2,085,000	Arizona.....	6,000
North Carolina.....	2,700,000	Washington.....	U.
South Carolina.....	412,000	Oregon.....	101,000
Georgia.....	607,000	California.....	332,000
Alabama.....	95,000	Nevada.....	U.
Mississippi.....	35,000		
Kentucky.....	1,330,000		
Tennessee.....	2,815,000		

SUMMARY

Plants of the Mediterranean and Red Fern wheat varieties moderately infected with leaf rust from tillering to maturity had their yields reduced 63.3 and 57.2 per cent, respectively.

The yield of the Fulcaster variety, heavily infected from tillering to maturity, was reduced 97.4 per cent. Severe infection from shooting to maturity resulted in 91.3 per cent reduction. The yield of plants heavily rusted when the heads were just showing in the boot was reduced 54.3 per cent. The yield of plants heavily rusted in the period from blossoming to maturity was reduced 24.7 per cent.

The yield of Michigan Amber, heavily rusted when heads were emerging from the boot, was reduced 37.2 per cent. When 100 per cent infection was produced at blossoming, the yield was reduced 27.2 per cent in one case and 33.5 per cent in another.

By dusting with sulphur, leaf rust was greatly reduced in field plots. Undusted plots of the winter wheat Trumbull which became rusted from 50 to 100 per cent following blossoming yielded 11.1 per cent less than dusted plots. Undusted plots of the spring wheat Illinois No. 1 which became rusted 50 per cent at blossoming, increasing to 100 per cent later, yielded 24.2 per cent less than the dusted plots.

The leaves of rusted plants died in advance of the corresponding leaves on check plants, and the weight of the straw was decreased. In early infections the reduction was 70 per cent or more. Later infections reduced the weight of straw from 11 to 33 per cent, depending on the duration of the infection. In the case of the Michigan Amber variety, rust infection prevented purpling of the straw, suggesting a shortage of carbohydrates.

The yield of plants of the Webster variety, highly resistant to physiologic form 5, was reduced 11.4 per cent when they were abundantly infected during emergence of the heads from the boot. Although highly resistant, the leaves of infected plants died in advance of those of the checks. The reduction in yield was mostly due to slightly smaller and lighter kernels. Tests of resistant varieties adjacent to susceptible varieties probably do not indicate the entire yielding capacity possible when planted in large acreage.

Yield is reduced by the production of fewer and lighter kernels. Severe infections over long periods bring about a marked reduction in the number of kernels and also a decrease in the weight of kernels, whereas infections over shorter periods result largely in a decreased weight of kernels. Early infections increase the number of shriveled kernels. In late infections the kernels are slightly smaller and of less weight.

The reduction in number of kernels is correlated with relative time of blossoming. The late flowers, the flowers of the upper and lower spikelets, and the upper flowers of the central spikelets fail to develop kernels in the rusted plants to the same extent as in check plants.

Early, severe autumn infection may considerably increase the winterkilling of winter wheat.

Leaf rust usually is prevalent in the soft-winter-wheat area of the United States, and in most years it is an important factor in production. In the hard-winter-wheat and in the spring-wheat areas it frequently is not so severe as in the soft-winter-wheat area. In some years, as in 1926 in the hard-winter-wheat area and in 1927 in the spring-wheat area, it is a very important factor in wheat production.

LITERATURE CITED

- (1) BAILEY, D. L., and GREANEY, L. J.
1926. PRELIMINARY EXPERIMENTS ON THE CONTROL OF LEAF AND STEM RUSTS OF WHEAT BY SULPHUR DUST. (Abstract) *Phytopathology* 16: 64.
- (2) CARLETON, M. A.
1899. CEREAL RUSTS OF THE UNITED STATES: A PHYSIOLOGICAL INVESTIGATION. U. S. Dept. Agr., Div. Veg. Physiol. and Path. Bul. 16, 74 p., illus.
- (3) COBB, N. A.
1890-94. CONTRIBUTIONS TO AN ECONOMIC KNOWLEDGE OF AUSTRALIAN RUSTS (UREDINEÆ). *Agr. Gaz. N. S. Wales* 1: [185]-214; 3: 44-68, 181-212; 5: 239-252, illus.
- (4) ERIKSSON, J., and HENNING, E.
1896. DIE GETREIDEROSTE IHRE GESCHICHTE UND NATUR SOWIE MASS-REGELN GEGEN DIESELBEN . . . BERICHT ÜBER DIE AM EXPERIMENTALFELDE DER KGL. SCHWEDISCHEN LANDBAU-AKADEMIE IN DEN JAHREN 1890-93 MIT STAATSUNTERSTÜTZUNG AUSGEFÜHRTE UNTERSUCHUNG. 463 p., illus. Stockholm.
- (5) GROVE, W. B.
1913. THE BRITISH RUST FUNGI (UREDINALES), THEIR BIOLOGY AND CLASSIFICATION. 412 p. Cambridge.
- (6) KIESSELBACH, T. A.
1925. WINTER WHEAT INVESTIGATIONS. *Nebr. Agr. Expt. Sta. Research Bul.* 31, 149 p., illus.
- (7) KIGHTLINGER, C. V.
1925. PRELIMINARY STUDIES ON THE CONTROL OF CEREAL RUSTS BY DUSTING. *Phytopathology* 15: 611-613.
- (8) ——— and WHETZEL, H. H.
1926. SECOND REPORT ON DUSTING FOR CEREAL RUSTS. (Abstract) *Phytopathology* 16: 64.
- (9) KLEBAHN, H.
1914. KRYPTOGAMENFLORA DER MARK BRANDENBURG. PILZE III—UREDINEEN. *Kryptogam. Brandenb.* 5a: 69-904, illus.
- (10) MCALPINE, D.
1906. THE RUSTS OF AUSTRALIA. THEIR STRUCTURE, NATURE, AND CLASSIFICATION. 349 p., illus. Melbourne.
- (11) MAINS, E. B., and JACKSON, H. S.
1926. PHYSIOLOGIC SPECIALIZATION IN THE LEAF RUST OF WHEAT, *PUCCINIA TRITICINA* ERIKSS. *Phytopathology* 16: [89]-120, illus.
- (12) MELCHERS, L. E.
1917. *PUCCINIA TRITICINA* ERIKSS. LEAF-RUST OF WINTER WHEAT CAUSES DAMAGE IN KANSAS. *Phytopathology* 7: 224.
- (13) REED, H. S., and COOLEY, J. S.
1913. THE EFFECT OF THE CEDAR RUST UPON THE ASSIMILATION OF CARBON DIOXIDE BY APPLE LEAVES. *Va. Agr. Expt. Sta. Ann. Rpt.* 1911-12: 91-94, illus.
- (14) ROEBUCK, A., and BROWN, P. S.
1923. CORRELATION BETWEEN LOSS OF LEAF AND DAMAGE TO CROP IN LATE ATTACKS ON WHEAT. *Ann. Appl. Biol.* 10: 326-334, illus.
- (15) TEHON, L. R.
1927. EPIDEMIC DISEASES OF GRAIN CROPS IN ILLINOIS, 1922-1926. THE MEASURE OF THEIR PREVALENCE AND DESTRUCTIVENESS AND AN INTERPRETATION OF WEATHER RELATIONS BASED ON WHEAT LEAF RUST DATA. III. *Natl. Hist. Survey Bul.* 17: 1-96, illus.
- (16) WEAVER, J. E.
1916. THE EFFECT OF CERTAIN RUSTS UPON THE TRANSPIRATION OF THEIR HOSTS. *Minn. Bot. Studies* 4: [379]-406, illus.

LEAF SPOT AND FOOT ROT OF KENTUCKY BLUEGRASS CAUSED BY *HELMINTHOSPORIUM VAGANS*^{1 2}

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INTRODUCTION

In an abstract³ published in 1922 the writer reported the widespread occurrence on Kentucky bluegrass (*Poa pratensis* L.) of a leaf spot caused by a species of *Helminthosporium*. Often, especially in moist situations, the lesions had been found on the leaf sheaths at the base of the plants as well as on the blades, giving rise to a condition comparable to foot rot of wheat. A brief account of the parasite, containing a discussion of its distribution and its pathological effects in addition to its description under the name *Helminthosporium vagans* Drechs., was included the following year in a paper⁴ dealing with various forms parasitic on grasses. Except for a report by Gardner⁵ stating that leaf spot of June grass due to *H. vagans* was prevalent at La Fayette, Ind., during May, 1923, the literature seems to offer no information concerning the disease in which the parasite involved was referred to by the binomial mentioned. However, Monteith⁶ reported that the leaf-spot disease of bluegrass had been destructive in fairways of golf courses in New Jersey and Pennsylvania during the early part of June, 1924. Although in the text the causal fungus was not cited by name, its identity was indicated in the statement that it was "closely related to those causing stripe, net blotch, spot blotch, and similar serious diseases of various grain crops." In a second paper⁷ by the same writer extensive browning of bluegrass caused by the same leaf spot was noted as having occurred during May of the following year on some golf courses in the vicinity of Washington, D. C., and on the plots at the Arlington Experiment Farm, Rosslyn, Va. While the illustrations of severely affected leaves accompanying the text of the latter account appear altogether characteristic of those attributable to *H. vagans*, it may not be amiss to add that specimens of the diseased grass that formed the basis of Monteith's two reports had been submitted to the present writer for examination and the cause of the trouble had been determined as the parasite under consideration by microscopic inspection of the conidiophores and conidia present on the better developed lesions.

At the time the description of *Helminthosporium vagans* was drawn up⁸ the writer's observations were limited to the development of the fungus on Kentucky bluegrass growing in fields or in lawns cut only

¹ Received for publication Oct. 12, 1929; issued March, 1930.

² A popular summary of this paper appeared in the following publication: DRECHSLER, C. LEAF SPOT AND FOOT ROT OF BLUEGRASS. Bul. Green Sect. U. S. Golf Assoc. 9: 120-123, illus. 16-20.

³ DRECHSLER, C. A NEW LEAF SPOT OF KENTUCKY BLUEGRASS CAUSED BY AN UNDESCRIBED SPECIES OF *HELMINTHOSPORIUM*. (Abstract.) Phytopathology 12: 35. 1922.

⁴ DRECHSLER, C. SOME GRAMINICOLOUS SPECIES OF *HELMINTHOSPORIUM*: I. Jour. Agr. Research 24: 641-740, illus. 1923.

⁵ GARDNER, M. W. INDIANA PLANT DISEASES, 1923. Ind. Acad. Sci. Proc. (1924) 34: 297-313, illus. 1925.

⁶ MONTEITH, J., JR. THE LEAF-SPOT DISEASE OF BLUEGRASS. Bul. Green Sect. U. S. Golf Assoc. 4: 172-173. 1924.

⁷ MONTEITH, J., JR. LEAF SPOT OF BLUEGRASS. Bul. Green Sect. U. S. Golf Assoc. 5: 198-199, illus. 1925.

⁸ DRECHSLER, C. Op. cit., p. 687. (See footnote 4.)

at moderate intervals and then not excessively close. Although some material collected in Brooklyn, N. Y., in August, 1920, showed leaves that had withered as a consequence of infection, no instances of the killing of plants down to the ground had come to light. While, therefore, it was apparent that "under certain conditions of moisture and of temperature such as would favor a multiplication of foliar lesions and accentuate the foot-rot symptoms the damage may not be altogether unappreciable," the sparse scattering of lesions ordinarily displayed was held to mark the parasite as one of minor destructiveness. Later observations made, especially in fairways of golf courses, where more rigorous cutting is practiced, dictate a modification of this view. As the outward expression of the disease and its relation to the causal fungus still seem far from being generally recognized even among students dealing with troubles affecting the grasses, it may not be inappropriate to amplify the somewhat inadequate account previously submitted.

EFFECT OF THE PARASITE ON THE HOST

LEAF SPOT

In the earlier paper the foliar lesions were described as being of the spot-blotch type, appearing as areas of bluish-black coloration, diminishing in intensity about the margin. Such lesions are indeed found in all representative lots of material, but where conditions have been favorable for the development of the trouble they are outnumbered by morbid regions mostly of larger dimensions. The central areas of these regions are bleached to a straw color, leaving the bluish-black or dark-brown discoloration restricted to a marginal zone of varying width. As the lesions of the latter category, which manifestly conform to the eyespot type, bear conidiophores and conidia in the central bleached parts, the presumption follows that they represent the more advanced stages in the development of the spot-blotch lesions. The correctness of this presumption was proved when cultures obviously belonging to the same identical fungus were obtained on agar plates planted with excised spot-blotch lesions, with excised peripheral portions of eyespot lesions, and with bits of agar media from dilution plates each containing a single conidium from an eyespot lesion after germination. Indeed, the obvious transition of one type of lesion into another, exhibited in a collection of leaves like that shown in Figure 1, A-M, provided visible evidence scarcely less conclusive than the more formal proof by comparison of fungus cultures isolated from them.

A feature frequently noticeable in leaves of *Poa pratensis* affected with leaf spot is a symmetrical development or symmetrical pairing of lesions relative to the midrib. Manifestly, such arrangement is traceable to infection at an early stage in the growth of the leaf while the latter is still tightly folded, the fungus mycelium then permeating the double thickness of folded blade regardless of anatomical relationships. With the unfolding of the leaf the infected mass is naturally separated into corresponding halves. The symmetrical or paired disposition was thus more frequent than usual following the early severe outbreak of the disease in April, 1929, as is evident in Figure 1, which represents leaves collected in the District of Columbia, April 30, from a stand that had been allowed to grow up unmolested and was heading out at the time.

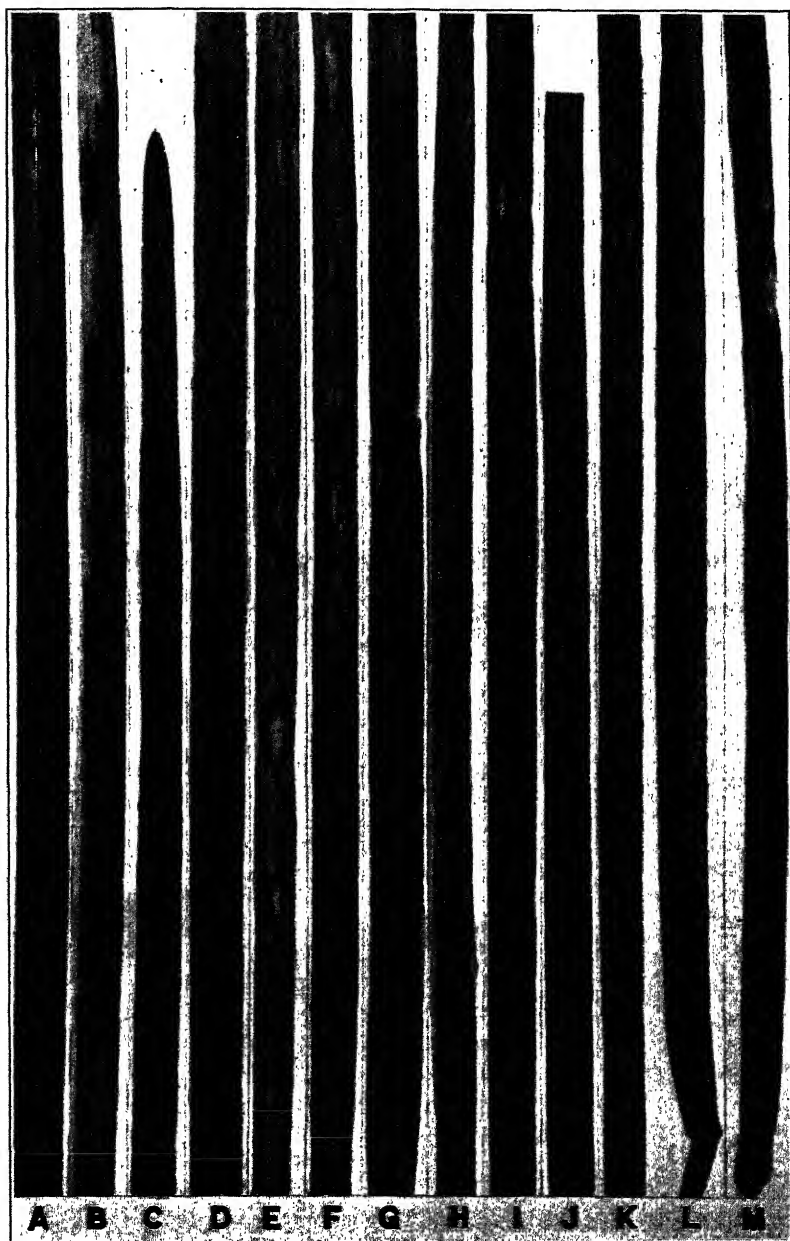


FIGURE 1.—A-M. Leaves of *Poa pratensis* affected with leaf-spot lesions due to *Helminthosporium vagans*. Collected April 30, 1929, in the District of Columbia, in a stand that had not been subjected to cutting. $\times 2$

FOOT ROT

Even in bluegrass plants left to grow to full size, leaf-spot lesions occur at the bases as well as in other parts of the leaf blades. (Fig. 1, L and M.) As the leaves are relatively broad under such conditions, a single lesion rarely occupies the entire width, so that the organ as a whole remains alive. In lawns cut close and at frequent intervals a much more delicate habit of growth is imposed on the host. A half dozen leaves greatly reduced in size are crowded into an almost rosettelike arrangement. Lesions of only moderate width may extend entirely across the basal part of the blade, thus bringing about its eventual death. (Fig. 2.) A more serious situation results when infection spreads to the closely imbricated delicate leaf sheaths that make up the upper portion of the central axis of the plant. All of the foliar organs, including the very young structures enveloped within, are invaded by the fungus, with the result that the miniature plant is effectually destroyed. (Fig. 3.) In April, 1929, the fairways in the public golf courses in the District of Columbia showed numerous gray or brownish patches in which such destruction was so general that the turf survived only in scattering plants and isolated stools.

That the severe manifestation of the disease in the fairways was caused not only by the prevalence of the parasite, but also by the excessively close cutting to which the grass was subjected, became evident from the fact that the same host in the rough near by, treated less rigorously, appeared in relatively good condition. To be sure, the foliage bore an abundance of lesions, and more than a few leaves were cut off by injuries at the base of the blade, but only occasional plants were killed entirely, and the general appearance of the turf was not noticeably impaired. Undoubtedly, close cutting operates to the disadvantage of the host, not only by imposing a delicate habit of growth especially subject to serious injury by the parasite, but also by entailing an excessive removal of foliage and a consequent loss in capacity for renewed growth. Owing to its generally erect foliar habit, bluegrass is shorn of its leaves in far more rigorous measure than are turf grasses of a more nearly prostrate leaf habit, like creeping bent (*Agrostis stolonifera* L. var. *compacta* Hartm.). The recommendation made by Monteith that when leaf spot is prevalent on Kentucky bluegrass in golf courses the blades of the mower be raised as high as circumstances will permit appears, therefore, to be thoroughly sound.

THE PARASITE

CULTURAL CHARACTERISTICS

As has been suggested previously, *Helminthosporium vagans* can be isolated without difficulty by excising bits of infected host tissue and, after surface sterilization, planting them on a suitable medium like maize-meal agar. Conidia may also be successfully utilized, in spite of the somewhat slow rate of mycelial extension characteristic of the fungus and the greater difficulty incurred thereby from the presence of contaminating bacteria. In cultural characteristics the fungus presents nothing striking enough to set it apart from congeneric types. Zonation involving both aerial and submerged mycelium is not uncommon (fig. 4, A), the darker submerged growth being composed of dark-brown filaments consisting of unusually strongly inflated segments. The tendency toward the formation of densely ramifying

systems, referred to in the earlier account, is emphasized by its growth on Dox agar⁹ illustrated in Figure 4, B, and would seem to be associated with retarded growth. That the quantity and the coloration of aerial mycelium is subject to considerable variation not readily traced



FIGURE 2.—A stool consisting of three plants of *Poa pratensis* vigorously attacked by *Helminthosporium vagans*. Many of the leaves are already killed, and those still green bear lesions varying in extent. Photographed from material collected April 30, 1929, in the District of Columbia from a golf course that had been subjected to excessively rigorous cutting. $\times 3$

to any specific cause is shown in Figure 4, C, *a* and *b*, representing two growths obtained in parallel potato-dextrose agar plate cultures. In tube cultures, owing apparently to the moister conditions, flesh-colored columnar structures somewhat resembling those formed by *H. teres* Sacc. and *H. avenae* Eidam frequently make their appearance.

⁹ Distilled water, 1,000 c. c.; magnesium sulphate, 5 gm.; dipotassium phosphate, 1 gm.; potassium chloride, 0.5 gm.; ferrous sulphate, 0.01 gm.; sodium nitrate, 2 gm.; dextrose, 5 gm.; agar, 15 gm.

MORPHOLOGY AND TAXONOMY

Although in artificial culture *Helminthosporium vagans* generally remains sterile, occasionally a moderate number of conidiophores and conidia are produced. In some instances the sporophores are represented by the terminal portions of aerial hyphae differentiated somewhat from the vegetative parts by a slightly greater diameter, a

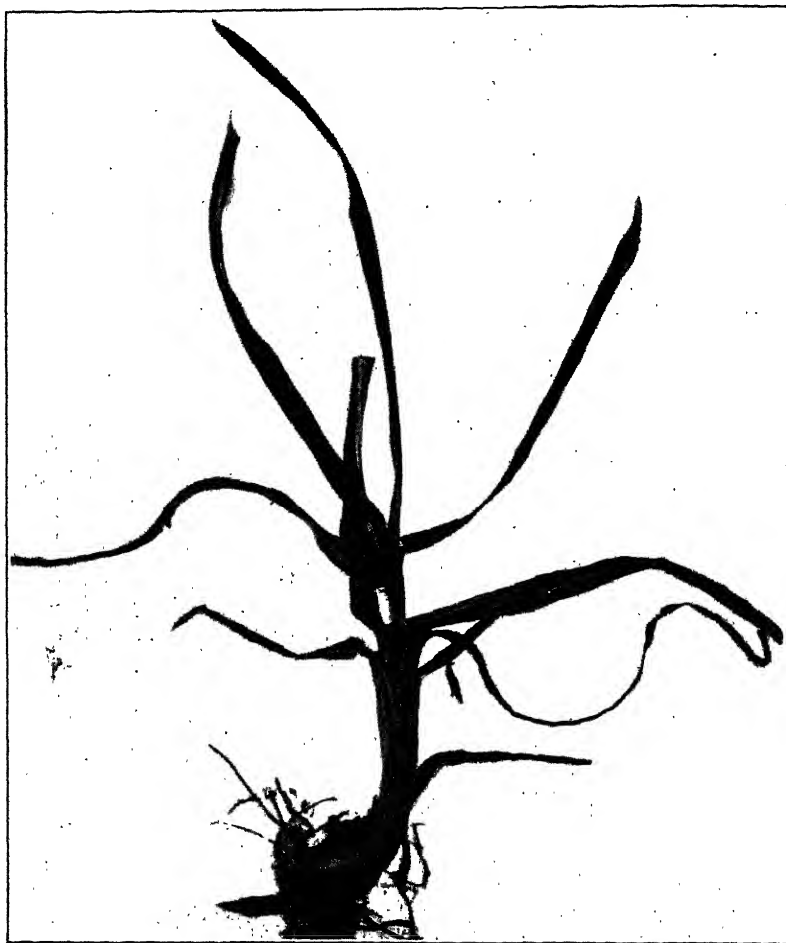


FIGURE 3.—A plant of *Poa pratensis* severely attacked by *Helminthosporium vagans*. The three remaining green leaves are so severely affected at the closely arranged sheaths as to leave no doubt as to their early destruction. Photographed from material collected April 30, 1929, in the District of Columbia, from a golf course that had been subjected to excessively rigorous cutting.

X 3

thicker wall, and a deeper coloration. More frequently, however, they are present as lateral branches on the longer aerial filaments, modified in the same manner as the terminal conidiophores, the deeper coloration and greater thickness of wall usually extending a short distance into one or both arms of the parent hypha. (Fig. 5, A-H.) These lateral sporophores ordinarily show a range in length from approximately 25μ (fig. 5, C) to over 100μ (fig. 5, G), and a range in

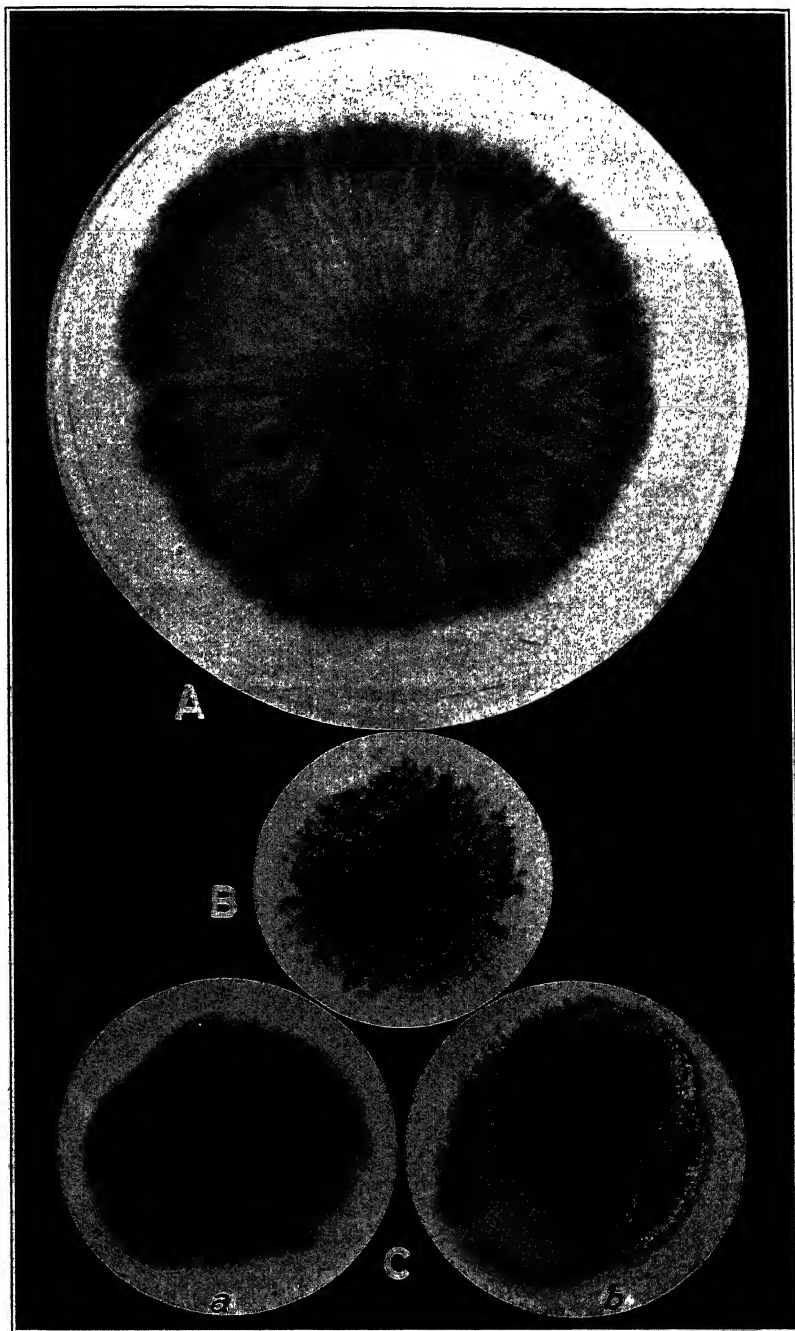


FIGURE 4.—Growth of *Helminthosporium vagans* in plate culture 12 days after planting: A, Maize-meal agar; B, Dox agar; C, potato-dextrose agar. The ready variability of the fungus with respect to the appearance of the aerial mycelium is shown in C, *a* and *b*, representing parallel cultures of the same age and on the same substratum. $\times 1$

number of septa from one (fig. 5, C, and H, *a*) to five (fig. 5, G). Most conidiophores give rise to only one conidium (fig. 5, A-E), but instances in which continued development results in the production of a second spore are not rare (fig. 5, F, *a, b*, and G, *a, b*). The lateral sporophores are usually inserted on the parent filament at rather long intervals, though cases of more closely approximated origins occur. (Fig. 5, H, *a* and *b*.) The conidia produced in culture on artificial media resemble in general shape those found in nature. With respect to coloration they are generally somewhat darker, because of a larger proportion of olivaceous individuals than yellowish or brownish ones. Especially marked, however, is their inferiority in size and number of septa, as is evident by a comparison of the conidia shown in Figure 5, A-H, with those shown in Figure 5, I-S, the latter having been drawn at the same magnification from field material. Such inferiority, to be sure, is generally characteristic of most of the species of *Helminthosporium* parasitic on grasses that can be induced to sporulate at all in artificial culture.

In this connection attention is directed to certain of the more extreme expressions of morphological features pertaining to conidia produced under natural conditions. The conidium represented in Figure 5, L, for example, exhibits 12 septa, 2 in excess of the number allowed for in the original diagnosis. The one shown in Figure 5, J, was found to measure 137μ in length, while the spores represented in Figure 5, P and S, measured 25μ in diameter—magnitudes also somewhat in excess of those previously submitted. In order that undue importance may not be assigned to such extreme values, it may not be amiss to include here a brief summary of measurements of lengths and diameters made on 200 living conidia selected at random in mounts of material scraped from Kentucky bluegrass leaves collected on a golf course in the District of Columbia on May 1, 1929. The 200 measurements of spore lengths fall into classes covering range intervals of 5μ , as follows: 31–35 μ , 3; 36–40 μ , 3; 41–45 μ , 4; 46–50 μ , 6; 51–55 μ , 3; 56–60 μ , 4; 61–65 μ , 14; 66–70 μ , 13; 71–75 μ , 21; 76–80 μ , 18; 81–85 μ , 25; 86–90 μ , 26; 91–95 μ , 17; 96–100 μ , 13; 101–105 μ , 11; 106–110 μ , 7; 111–115 μ , 2; 116–120 μ , 5; 121–125 μ , 2; 126–130 μ , 1; 131–135 μ , 0; 136–140 μ , 2. The corresponding 200 measurements of spore diameters are referable to the different values found, as follows: 11 μ , 1; 12 μ , 1; 13 μ , 1; 14 μ , 1; 15 μ , 4; 16 μ , 5; 17 μ , 25; 18 μ , 40; 19 μ , 41; 20 μ , 38; 21 μ , 19; 22 μ , 10; 23 μ , 8; 24 μ , 3; 25 μ , 3. Counts of cross walls made on the same 200 individual conidia are distributable as follows: 2 septa, 3; 3 septa, 8; 4 septa, 21; 5 septa, 34; 6 septa, 39; 7 septa, 46; 8 septa, 28; 9 septa, 14; 10 septa, 4; 11 septa, 1; 12 septa, 2. Computations from the three sets of values for the 200 spores gave 82.7 μ as the average length of the conidium, 19.1 μ as the average diameter, and 6.3 as the average number of septa. The conidium shown in Figure 5, I, thus represents a moderately close approximation to the average values determined from field material, though comparing more than favorably with the largest individual spores produced in artificial culture.

While conidia of members of genus *Helminthosporium* do not regularly exhibit septa other than transverse cross walls, oblique and even longitudinal partitions occur rather sparingly in most species. Such departures from the usual morphological trend are present also in conidia of *Helminthosporium vagans*. Oblique partitions are not

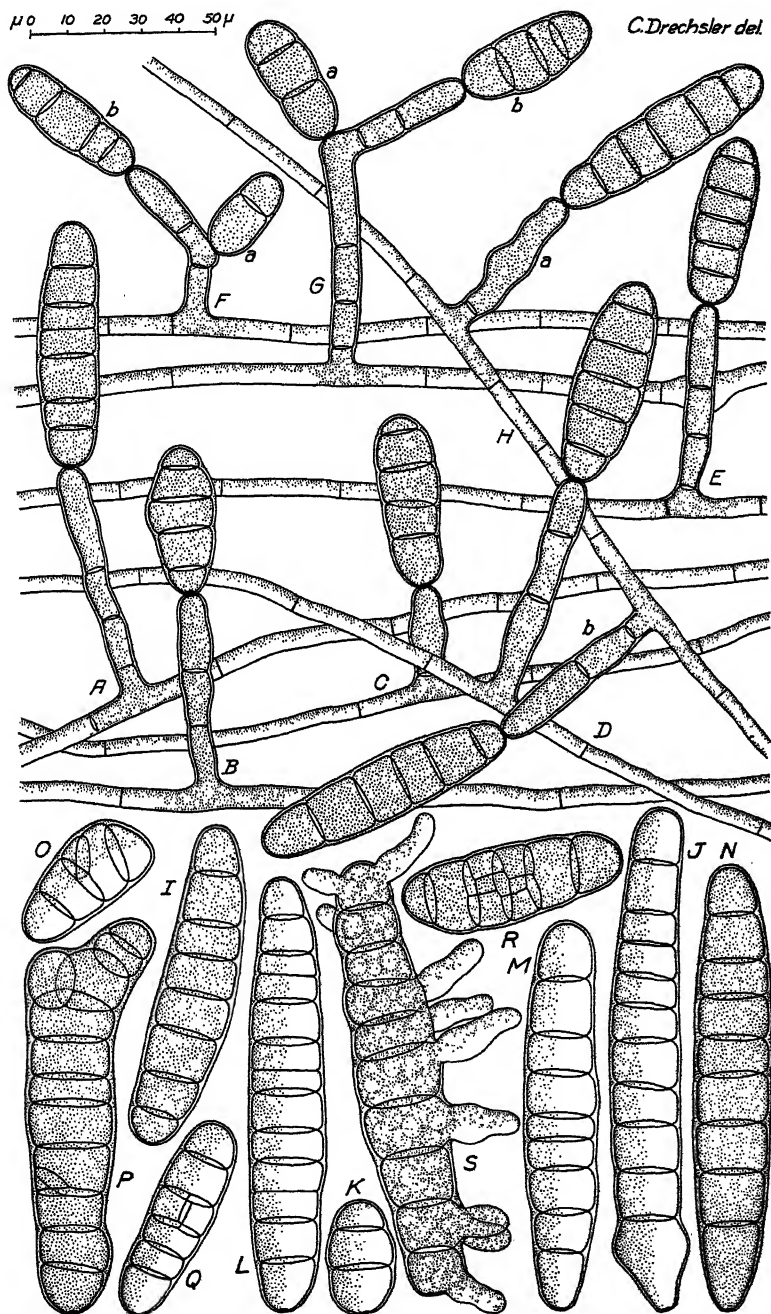


FIGURE 5.—A-H, Portions of aerial filaments of *Helminthosporium vagans* bearing conidiophores with one or two conidia. Drawn from material produced in a 30-day-old maize-meal agar plate culture. $\times 500$. I-S, Conidia of *H. vagans* from affected leaves of *Poa pratensis* collected in Washington, D. C., May 1, 1929. Note the germination of S by the production of germ tubes from intermediate as well as from end segments. $\times 500$

infrequently associated with marked curvature in the axis of the spore, though in many instances their presence, like that of longitudinal septa for the most part, is independent of any unusual external feature. The occurrence of one or two septa of the latter type, especially in conidia of the smaller dimensions, brings about a striking resemblance to ascospores of certain species of *Pyrenophora* having a *Helminthosporium* stage parasitic on grasses. The resemblance is of interest especially because the germination of the conidia by the production of germ hyphae indiscriminately from the intermediate as well as from the end segments (fig. 5, S) indicates that *H. vagans* is indeed to be regarded as belonging to that series of forms achieving their sexual stage in the ascigerous genus mentioned, or in the very closely related, if not identical, genus *Pleospora* Fries. That the muriformly septate bodies in question were actually conidia rather than ascospores was sufficiently apparent in that they were regularly provided at one of the ends with a scar entirely like the basal scar invariably present in the more regularly partitioned conidia of the leaf-spot fungus, and marking the point of their former attachment to the conidiophore.

Owing to the exceptionally early appearance of the leaf spot on Kentucky bluegrass in 1929, with the resulting occurrence of the causal parasite in quantity on new growth while the withered remains of growth of the previous season were still intact, opportunity for the discovery of an ascigerous stage of *Helminthosporium vagans* might well be considered as having been favored in at least one respect. Some effort was therefore made to detect perithecia that might plausibly represent a phase in the life history of the parasite. However, no perithecial form occurring in a relationship suggesting an ontogenetic connection was found in any of the decaying material examined.

SUMMARY

Helminthosporium vagans, previously set forth as a relatively innocuous parasite on *Poa pratensis*, has at various times during several seasons been found to cause conspicuous damage, especially in the fairways of golf courses. The most destructive effects occur apparently when the grass has been subjected to close cutting. The delicate habit of growth encouraged thereby results in plants with the upper axial part consisting mostly of imbricated leaf sheaths of such small proportions as to be subject to direct destruction through the development of foot-rot lesions. The excessive reduction of functional leaves resulting from close clipping because of the erect foliar habit of the grass evidently reduces the capacity for renewed growth. The few field observations made by the writer confirm the validity of the recommendation of less drastic mowing as preventive and remedy.

In pure culture the fungus occasionally produces conidia resembling the smaller ones produced under natural conditions, the conidiophores being represented by noticeably differentiated terminal prolongations or lateral branches of aerial filaments. The germination of the conidia by germ tubes arising from middle as well as from end segments indicates close affinity of the fungus with the series of graminicolous congeners achieving their ascigerous phase in the genus *Pyrenophora*, although no sexual stage of any kind has yet been found associated with it.

WOOL FINENESS AS INFLUENCED BY RATE OF GROWTH¹

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INTRODUCTION

A study of the variations in the fineness of individual wool fibers as they grow has revealed facts that are of practical significance to the flockowner and the wool trade. According to Roberts² "fineness is one of the most important properties of wool." Fleeces with good, sound, uniform fibers bring a better return to the woolgrower and are more desired by the wool manufacturer than those showing undesirable irregularities in diameter along their length. Such well-grown, uniform fleeces make possible the production of cloth of better quality than is possible with similar fleeces produced under poor growing conditions.

Close examination of a large number of fleeces under varying conditions of sheep management has led to the belief that the fineness of wool fibers throughout the growing period of a fleece is affected by the conditions of feeding, breeding, and management under which the wool has been grown. Testing for strength of staple with the fingers has indicated that there is considerable difference in fleeces that appear to be of the same fineness. One often finds wool at shearing time that is weak at some point in the staple and will break under a slight strain. This condition of the wool may have been caused by sickness or other adverse conditions, which in many cases might have been avoided by better management of the sheep.

Roberts³ refers to the "rise" or thinning of fibers which takes place just before shearing as an intermediate condition of shedding. When this condition occurs it may be caused by hereditary factors. A wool merchant may buy a considerable quantity of staple wool and find many fleeces in the lot that are weak, a condition referred to by the trade as "tender." When these wools are tested for staple strength they yield quickly at one well-defined line across the staple. A small staple is often so weak in one place that, when held horizontally, it will bend over from its own weight. The wool from these tender fleeces is not suitable for combing and the fleeces are, therefore, usually put in with the clothing grades. Sometimes this weak or tender place in the wool occurs a short time before shearing and at a point so near the end of the wool fibers that there is still sufficient length of wool to comb into tops. However, there is a smaller yield of long fibers from these fleeces and they are, therefore, not so profitable to the manufacturer.

¹ Received for publication Oct. 5, 1929; issued March, 1930.

² ROBERTS, J. A. F. WOOL RESEARCH AND THE FARMER. Brit. Research Assoc. for Woollen and Worsted Indus. Pub. 103, 16 p. 1928.

³ ROBERTS, J. A. F. Op. cit., p. 10.

PRELIMINARY OBSERVATIONS

In the fall of 1924 the senior writer visited a number of wool houses in Boston and obtained samples of tender wool in order to secure information that would help to overcome this defect. In a study of these samples a thinning out of the fibers was readily observed, but there was difficulty in finding a tender place in a fiber that was entirely within the field of the microscope when it was magnified about 150 diameters. A sample of wool obtained from a very tender Australian fleece had an abrupt change in the diameter of the fibers. Figure 1 illustrates such a weak place or "break" in the sample, and

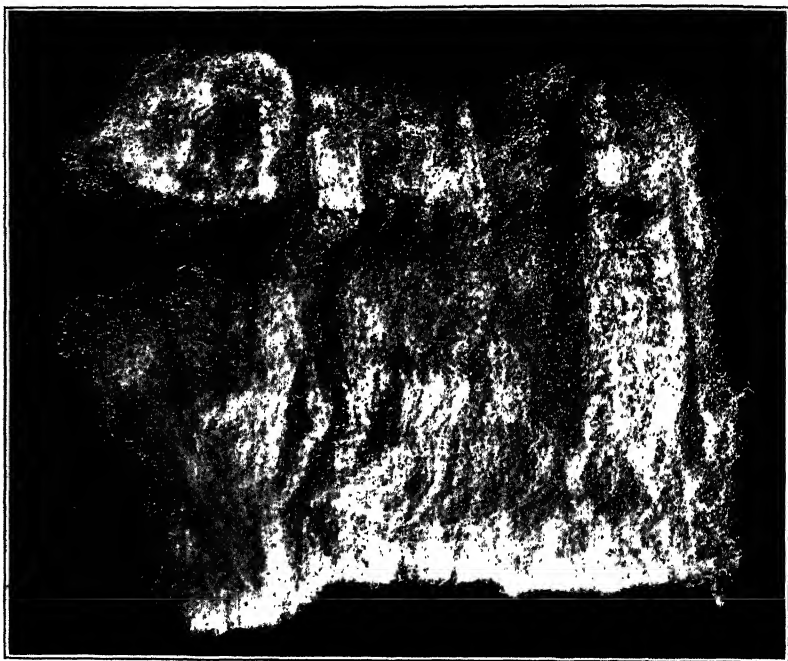


FIGURE 1.—A "break" in a staple. The wool is partially pulled apart to show the location of the weak spot. Approximately natural size

Figure 2 is a microphotograph of fibers from the same fleece. These fibers, which are tender and have a very small diameter at one point, show no indications of having ceased to grow.

Weak places in staple wool may be evident in varying degrees according to the reduction in the diameter of the fibers during the period the weak portion of the fiber was growing. Sometimes a sheep may be subjected to such severe conditions that the wool appears to stop growing for a short time. Later, when the sheep becomes thrifty, new fibers apparently are formed in the wool follicle. As these new fibers grow they become embedded in the old ones. This condition is shown in Figure 3. The old fiber will separate from the new fiber under the slightest strain. The sample from which this fiber was taken was obtained from a Southdown sheep seriously infested with internal parasites. All the samples mentioned above, except the last, were warehouse samples of which the previous history

was not available, hence there was no direct information on the cause of formation of the tender wool.

EXPERIMENTAL PROCEDURE

METHOD OF OBTAINING SAMPLES

In order to study wool fineness as influenced by the wool's rate of growth, the writers selected five healthy Corriedale ewes all of which

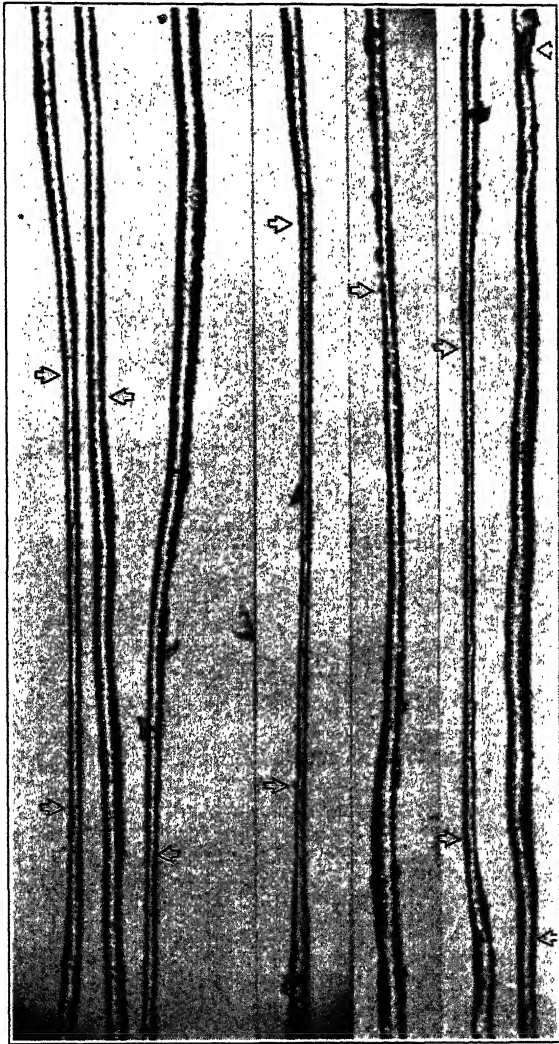


FIGURE 2.—Photomicrograph of fibers taken from lock of wool shown in Figure 1. The weak places in the fibers which have noticeably reduced diameters are indicated by arrows. $\times 140$

produced lambs each year with the exception of sheep 1125 in 1928. The ages of these sheep varied from 1 to 4 years at the beginning of this experiment. Under the supervision of C. G. Potts, of the bureau, these sheep were weighed individually every two weeks and an accurate record was kept of their breeding and feeding.

Wool-growth clippings were obtained from these sheep at 28-day intervals, in the following manner: All the fibers were removed from an area about one-half inch square, located about 3 inches to the rear of the point of the shoulder and midway between back and belly. This area was selected since previous study had shown wool from this portion to be fairly typical of the entire fleece. Twenty-eight days later two small samples of wool, representing the growth during that period, were taken from the same area. The remaining fibers on the area were removed with sharp scissors that cut very close to the skin. The operation was repeated every 28 days. On the same dates that fiber clippings were taken, two small locks of wool were tied with dental floss close to the skin on the right and left side of the same

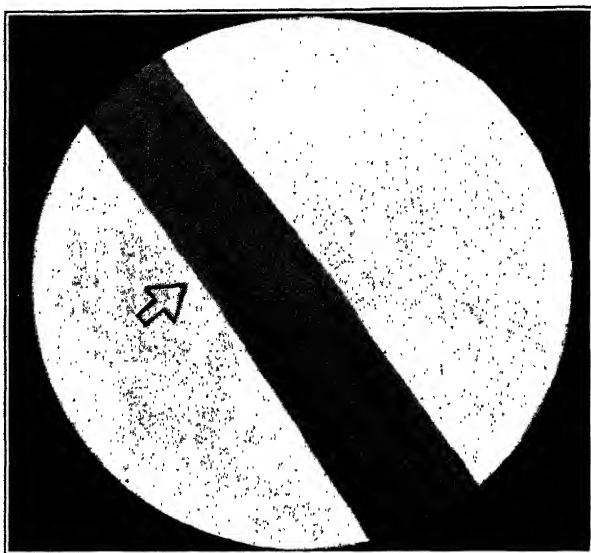


FIGURE 3.—Photomicrograph of the juncture between new and old growths in a wool fiber; the fiber is very weak at such places. \times about 500

sheep. Figure 4 illustrates the appearance of the tied locks. These locks made it possible to study the growth of individual wool fibers during successive 28-day periods.

LABORATORY METHODS OF MEASUREMENT

The lengths of the wool clippings were measured with a binocular microscope magnifying 7.5 diameters. This was accomplished by first placing a few of the fibers on a piece of thin glass beneath which was a sheet of cross-section paper of the same size, ruled in millimeters. A microscope slide was then placed across the fibers even with one of the lines of the cross-section paper. The fibers were then pulled slowly out from beneath this slide with small tweezers. When a fiber was just free from the microscope slide, the movement of the tweezers was stopped and the length of the fiber was read to the nearest millimeter. Five fibers were measured from the left and five from the right side of each sheep. The diameter of the wool fibers was meas-

ured with a micrometer caliper having a ratchet stop and graduated to one four-hundredth of a millimeter.



FIGURE 4.—Tied locks showing one year's growth of wool from Corriedale sheep by 28-day intervals. Actual size

Fifty fibers were used for diameter measurements from clippings and tied locks from the left side of each sheep, and 50 from the right. This number was considered sufficiently large to furnish a repre-

sentative average of the samples. Portions representing the same growth period of 25 fibers from the left and from the right side samples

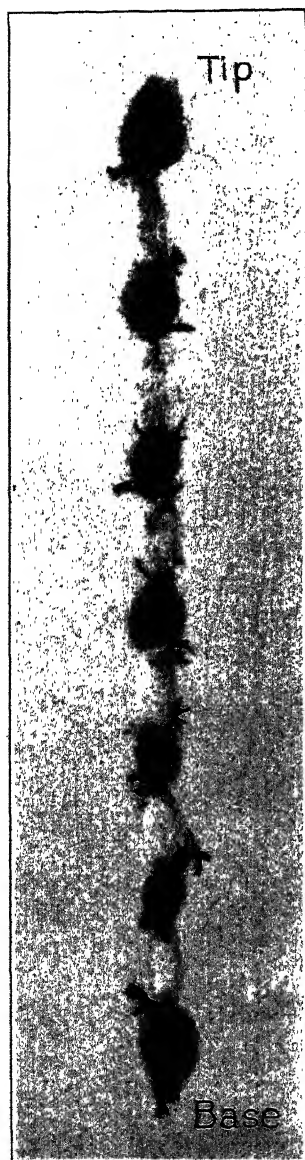


FIGURE 5.—Tied lock of wool with alternate portions dyed to show the different growth periods. Actual size.

of each sheep were measured both with the micrometer caliper and with a microprojector in order to compare the results by these two methods. Under the conditions, this number was considered sufficient to show an accurate comparison between the two methods. By means of the microprojector, the fibers were projected with a 1,000-diameter magnification on an aluminum-coated screen. The measurements on the screen were made with a scale graduated in millimeters. With this magnification each millimeter of the image, as projected on the screen, represented 1 micron of actual wool fiber.

The length and diameter of clippings from the greatest, least, and last growth periods of the year 1927-28 were again measured in order to study the relationship of length and diameter to weight. For this purpose 100 fiber clippings were taken from the left and right sides of each sheep. This larger number of fibers was used in order to have a quantity that could be weighed with accuracy. After the length of each fiber was determined, its diameter also was immediately measured with a micrometer caliper. The weights of each of these groups of 100 fibers were taken on a microbalance and the fibers were then placed in small gelatine capsules for safe keeping and future reference.

All measurements were made under controlled conditions of temperature and humidity with the exception of those taken by means of the microprojector. Owing to the heat caused by the operation of the microprojector, it was impossible to maintain a constant temperature while making these measurements. For all other measurements the temperature was maintained at 70° F. and the relative humidity was kept at 65 per cent.

The various growth periods on the tied locks were shown more clearly by dyeing alternate growth periods as illustrated in Figure 5. This was accomplished by wrapping a thread saturated in crude wool grease around the growth of alternate 28-day periods so that it covered the entire growth of such periods; the greased thread excluded the dye from the covered portions of the

lock. The entire lock was then dyed in an alcoholic solution of malachite green dye just long enough to stain all the fibers of the unprotected growth periods.

After the wool-growth clippings and tied locks were measured for length, tabulations were made of the data on the basis of average length growth for the five sheep from the periods in which the wool grew the greatest length, the least length, and also for the last growth period before shearing. These measurements, from samples obtained from each of the five animals for four successive years, are presented in Table 1. The average diameter of the growth clippings for the same periods is also given. In four cases the greatest and least growth periods for individuals are not the same as that for the average of the group. These variations were largely due to differences in the dates of lambing and other factors influencing the physical condition of the sheep. The coarsest fibers were produced each year during the periods of greatest growth and the finest during those of least growth.

TABLE 1.—Average length and diameter of 100 wool fibers, for the greatest, least, and last growth periods, for five Corriedale sheep during four successive years

Sheep No.	For greatest growth period, 28 days ended Aug. 28, 1925		For least growth period, 28 days ended Jan. 12, 1926		For period just before shearing, 28 days ended May 4, 1926		For greatest growth period, 28 days ended Oct. 20, 1926	
	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>
977.....	1.27	22.75	1.06	21.78	1.12	16.90	1.40	25.00
979.....	1.47	22.98	1.08	21.95	1.24	20.33	1.48	23.83
982.....	1.21	22.80	.92	19.78	1.18	20.98	1.19	26.18
1125.....	1.50	20.08	.90	17.70	1.50	19.98	1.49	22.95
2B.....	1.55	28.00	1.01	24.38	1.16	24.33	1.56	28.70
Average.....	1.40	23.32	.99	21.12	1.24	20.50	1.42	25.83

Sheep No.	For least growth period, 28 days ended Jan. 12, 1927		For period just before shearing, 28 days ended Apr. 6, 1927		For greatest growth period, 28 days ended June 29, 1927		For least growth period, 28 days ended Feb. 8, 1928	
	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>
977.....	1.18	23.95	1.12	21.48	1.46	25.58	1.10	18.13
979.....	1.14	23.90	1.20	23.68	1.37	26.68	.98	21.58
982.....	1.05	25.00	1.23	25.03	1.22	27.08	1.05	21.05
1125.....	1.24	22.08	1.27	22.88	1.35	26.28	1.15	20.25
2B.....	1.30	27.58	1.13	23.25	1.37	30.05	1.02	26.70
Average.....	1.18	24.50	1.19	23.26	1.35	27.13	1.06	21.54

Sheep No.	For period just before shearing, 28 days ended May 1, 1928		For greatest growth period, 28 days ended Oct. 17, 1928		For least growth period, 28 days ended Feb. 6, 1929		For period just before shearing, 28 days ended May 1, 1929	
	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>	<i>Cm.</i>	<i>Microns</i>
977.....	1.16	23.00	1.28	23.80	1.08	22.13	1.25	23.15
979.....	1.15	23.15	1.26	24.20	1.10	22.68	1.14	21.05
982.....	1.12	23.33	1.10	24.63	.88	21.33	1.09	23.08
1125.....	1.27	23.73	1.82	22.03	1.11	22.83	1.13	24.18
2B.....	1.38	27.88	1.85	29.68	.99	25.70	1.21	27.83
Average.....	1.22	24.22	1.26	24.87	1.03	22.93	1.16	23.86

TABLE 2.—Comparison of average diameters of 100 wool fibers from tied locks of five Corriedale sheep, measured with a micrometer caliper and also by a microprojector

Sheep No.	Average diameter of 100 wool fibers—					
	For greatest growth period, 28 days ended June 29, 1927, ^a when measured with—		For least growth period, 28 days ended Feb. 8, 1928, ^b when measured with—		For period just before shearing, 28 days ended May 1, 1928, ^c when measured with—	
	Micrometer caliper	Microprojector	Micrometer caliper	Microprojector	Micrometer caliper	Microprojector
	Microns	Microns	Microns	Microns	Microns	Microns
977.....	25.28	26.66	19.10	20.50	21.83	24.74
979.....	24.98	28.08	20.35	22.64	21.58	26.20
982.....	27.23	30.42	20.98	23.68	22.25	24.56
1125.....	25.80	29.32	17.58	23.12	23.68	28.56
2B.....	31.03	34.36	26.03	30.04	27.75	30.72
Average.....	26.86	29.77	20.81	24.00	23.42	26.96

^a Average length of clippings, 1.35 cm.
^b Average length of clippings, 1.06 cm.

^c Average length of clippings, 1.22 cm.

The measurements of diameter which were taken with a micrometer caliper and also with a microprojector are recorded in Table 2 and

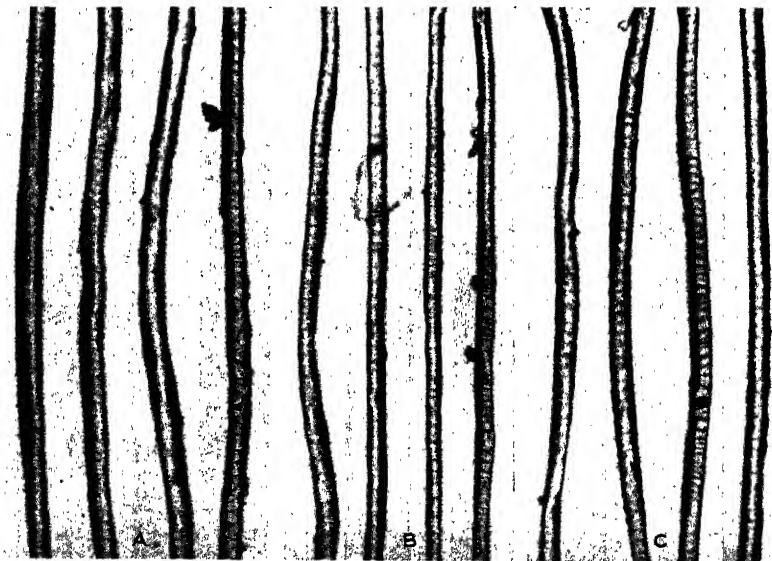


FIGURE 6.—Photomicrographs of four wool fibers as they appeared during their greatest (A), least (B), and last (C) growth periods. $\times 140$

Figure 6 is a microphotograph of four wool fibers showing portions of their greatest, least, and last wool growth periods. The measurements made with the micrometer caliper were consistently lower than those made with the microprojector. In order to determine the cause of these differences in the diameter measurements, 25 short pieces of fine uniform wire were measured by both methods. The average

diameter for the wire with the micrometer caliper was 82.80 microns and with the microprojector was 85.64, a difference of 2.84 microns. The average differences found between the measurements made with the micrometer caliper and the microprojector in the case of the wool fibers was 3.21 microns. Thus with both materials the measurement of fibers by means of the microprojector gave results slightly greater than those obtained with the micrometer caliper. The microprojector generally yields more consistent results, however, than the micrometer caliper, and makes possible the more rapid measurement of growth changes in the diameter of wool fibers. It is impossible to use the micrometer caliper for measuring changes occurring quickly because of the width of its jaws which are too large to measure short-time growth.

TABLE 3.—Average length, diameter, and weight of 100 wool-growth fiber clippings for the greatest, least, and last growth periods of four Corriedale sheep

Sheep No.	Item	For greatest growth period, 28 days ended June 29, 1927			For least growth period, 28 days ended Feb. 8, 1928			For period just before shearing, 28 days ended May 1, 1928		
		Length	Diameter	Weight	Length	Diameter	Weight	Length	Diameter	Weight
977	Left side.....	<i>Cm.</i> 1.248	<i>Microns</i> 24.95	<i>Mgm.</i> 1.20	<i>Cm.</i> 0.914	<i>Microns</i> 17.85	<i>Mgm.</i> 0.46	<i>Cm.</i> 1.036	<i>Microns</i> 21.88	<i>Mgm.</i> 0.73
	Right side.....	1.426	24.75	1.23	.984	16.70	.47	1.137	22.30	.77
	Average.....	1.337	24.85	1.24	.949	17.28	.465	1.087	22.09	.75
	Average weight of 1 cm. growth.....			.927			.490			.690
979	Left side.....	1.088	24.00	.94	.917	19.50	.51	1.040	21.50	.71
	Right side.....	.988	25.30	.80	.896	20.08	.57	1.090	23.00	.79
	Average.....	1.023	24.65	.87	.907	19.80	.54	1.065	22.25	.75
	Average weight of 1 cm. growth.....			.850			.595			.704
982	Left side.....	1.040	26.25	1.10	.840	18.50	.50	.910	20.00	.67
	Right side.....	1.090	26.50	1.25	.940	18.50	.54	.990	21.50	.79
	Average.....	1.065	26.38	1.175	.890	18.50	.52	.950	20.75	.73
	Average weight of 1 cm. growth.....			1.103			.584			.768
1125	Left side.....	1.250	25.00	1.170	.980	19.48	.64	1.090	24.85	.95
	Right side.....	1.300	25.75	1.34	1.040	18.95	.60	1.160	24.60	1.02
	Average.....	1.275	25.38	1.255	1.010	19.22	.62	1.125	24.73	.985
	Average weight of 1 cm. growth.....			.984			.614			.876
	Average.....	1.175	25.32	1.135	.939	18.70	.536	1.057	22.45	.804
	1 cm. growth.....			.964			.571			.760

The average of the length, diameter, and weight of 100 fibers from clippings taken from the left and right sides of four Corriedale sheep are shown in Table 3. The difference in weight of fiber between the greatest and the least wool growth periods for the four sheep was 52.8 per cent. As has been previously stated, the length of the wool-growth periods was 28 days, or one-thirteenth of a year. Based on a 10-pound fleece shrinking 55 per cent, this would mean a reduction of 0.182 pound of clean wool during a period of 28 days. The reduction in length of the wool from the greatest to the least growth period is 2.36 millimeters. For a reduction of 1 millimeter in length (with-

out taking diameter into consideration) there would be a reduction of 0.0771 pound of clean wool. In order to find out what proportion of the decrease in weight of fiber is due to the reduction in diameter, the weights of fiber for each period equivalent to 1 cm. growth were determined. These figures are given in the table opposite the average weight of 1 cm. The reduction in wool diameter from the greatest to the least growth period is 6.62 microns, with a corresponding reduction of 0.393 mgm. of wool fiber. This is equal to 6.11 per cent reduction in weight of wool fiber for each decrease of 1 micron in diameter. With these figures also based on a 10-pound fleece shrinking 55 per cent, there would be a reduction of 0.02 pound of clean wool in 28 days due to reduction in diameter of wool fiber. These data show that there is a reduction in weight of fleece resulting from both the reduced rate of growth and the reduction in fiber diameter.

This group of experimental sheep had better feed and care than most flocks of sheep and did not have to withstand the hardships of range conditions, yet the less favorable conditions in the winter months were reflected directly in their wool production. The wool of these sheep was sound, but not of such good quality as it would have been had it been uniform in diameter throughout its entire length. A reduced rate of growth associated with a reduction of the diameter of wool fiber is the first indication of tender undesirable wool.

DISCUSSION

An analysis of the records for these sheep showed that the greatest wool growth is associated with a thrifty condition of the sheep as indicated by the weight records. The end of the period of least growth of wool was found to be from about 45 days before lambing to about lambing time. After this fact was determined, the data for length of wool grown for the last period before shearing were selected and diameter measurements were made of fibers grown in this period. This was done in order to determine what the diameters of the fibers were during a period as late as possible before shearing.

Probst⁴ based a series of measurements on observations of Zorn who stated⁵ that wool grows two-thirds of its length in the first six to seven months and one-third of its length in the remaining months of the yearly period between shearings. On this basis, Probst measured a wool sample, from one of his stud bucks, at four different places along its length. He calculated that the measured portion of the tip was produced in August; that of the section next to the tip in October, before breeding time; the measured portion of the third section in December; and that of the base in March or April. He concluded that this decrease probably was due in part to the influence of the winter season and in part to reduced vigor of the buck after breeding time. The decrease, moreover, was more pronounced in 1923 than in 1924; Probst explains this by the fact that in the first instance the buck had run with the flock, while in the second case, hand breeding was practiced.

⁴ PROBST, E. DIE FEINHEITSBESTIMMUNG DES WOLLAARES. *Ztschr. Tierzucht u. Zuchtungsbiol.* 6: [403]-488, illus. 1926.

⁵ ZORN, W. HAUT UND HAARE ALS RASSE UND LEISTUNGSMERKMAL IN DER LANDWIRTSCHAFTLICHEN TIERZUCHT. *Flugschr. Deut. Gesell. f. Zuchtungsk.* 43, 1919. [Original not seen.]

The findings of Duerden and Bosman⁶ in their study of wool from seven sheep at three different places along the staple, including the top, middle, and base, show that "the growth of the fleece is less vigorous toward the end of the season than at any other period." It seems quite possible that the end of the season might have been unfavorable for the growth of the fleeces from which those seven samples were taken and that more favorable conditions would have been associated with a larger diameter. It is also possible that the reduced growth, in part, may have been caused by some hereditary factor. The writers' data on the clipping measurements also show that the average diameters for the greatest, least, and last growth periods vary directly with the average rate of growth.

SUMMARY

A study of five healthy Corriedale ewes was undertaken in order to determine, if possible, the effect of the rate of wool growth on fineness and length of fibers. Periodic wool-growth clippings and tied locks were used in this study. It was found that the rate of wool growth and the fineness of the fibers produced varied throughout the year, both growth and coarseness being greatest in summer or fall and least in midwinter. The greatest growth in length of fiber appeared to be correlated with the largest diameter of fiber and vice versa. The period of greatest wool growth was also associated with a generally thrifty condition of the sheep as indicated by their weight. The period of least wool growth occurred, in ewes, usually during lambing time and the 45 preceding days. The weight of the wool fibers increased as the length and diameter increased and vice versa. The indications are that the character of the fleece is probably very largely within the control of the flock owner because the experiments appear to show that there is a rather close relationship between the thriftiness of a sheep and the quality and quantity of wool it produces.

⁶ DUERDEN, J. E., and BOSMAN, V. ABSENCE OF UNIFORMITY IN GROWTH OF THE MERINO FLEECE. *Jour. Textile Inst.* 18: T191-T194, illus. 1927.



THE CHEMICAL COMPOSITION OF COLLOIDAL MATERIAL ISOLATED FROM THE HORIZONS OF VARIOUS SOIL PROFILES¹

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INTRODUCTION

Investigation of the chemical composition of colloidal material isolated from the soil has been confined largely to that occurring in the upper part of the soil profile; that is, in the A and B horizons. This work has indicated the extent to which soil colloidal material may vary in composition over a large area, namely, the humid portion of the United States, as well as in restricted areas of the same or related soils. It is important, however, especially in connection with the study of soil genesis and profile development that something be known of the composition of colloidal material in the lower part of the profile, in the C horizon, and in the zone of decomposing rock. Although colloids of the A and B horizons of most soils are of similar composition, it is not unlikely that greater differences might be found in colloids in the deeper parts of the profile where nature of the parent material rather than climatic influences would be expected to determine the composition of the colloidal material. Comparison of the composition of colloidal material isolated from the upper soil horizons with that occurring in the lowest horizons should indicate what changes have taken place in the colloid as the result of leaching and aging.

PREVIOUS WORK

The most extensive investigation of the composition of colloidal material isolated from the soil is that reported by Robinson and Holmes (9).² The investigations of others on the finest fractions of the soil have been reviewed by these writers and need not be considered here. Robinson and Holmes concluded from their study of the colloidal material in the A and B horizons of 15 American soils that the colloids isolated from the surface soil and from the corresponding subsoil are much alike in composition. More recently Holmes (4) reached the same conclusion from his study of colloids from the Leonardtown soil. McCool (8) has reported partial analyses of colloids from a group of Michigan soils which indicate rather wide variations in certain constituents in the different horizons. Potash appears to increase regularly with depth in the profile. Silica decreases regularly in most of the profiles studied, but in several

¹ Received for publication Nov. 15, 1929; issued March, 1930.

² Reference is made by number (italic) to "Literature cited," p. 482.

it increases regularly with depth. Phosphoric acid, lime, magnesia, and soda also show considerable variations which do not appear to follow any definite order.

METHODS

Colloidal material was isolated from the soil essentially as described by Robinson and Holmes and in other publications of this bureau. A diameter of 1 micron was taken as the upper limit of colloidal particles. The colloidal material was isolated quantitatively, and an effort was made to remove all the colloid present that could be dispersed by the method employed. The details of the process are as follows: A 200 to 400 gm. sample of soil material, depending on the quantity of colloid present, was worked by hand to a plastic condition and dispersed in about 50 liters of distilled water. The water was made alkaline to phenolphthalein by ammonia. The suspension was passed through a high-power centrifuge and the colloidal material was concentrated by drawing off the water through Pasteur-Chamberland filter candles. The residue in the centrifuge was dispersed in water as before and centrifuged, the process being repeated until a dispersion of the material in 3 liters of water gave after centrifuging only a slightly opalescent solution. In most cases this final colloidal solution contained 0.1 gm. or less of solid material. In other cases it seemed impossible to reach quite this lower limit. The concentrated colloid was evaporated to dryness on the steam bath.

The isolated colloids and their corresponding soils were analyzed by the fusion method as given by Hillebrand (3). Combined water was estimated by deducting from the loss on ignition the organic matter, which was determined as CO_2 by the dry-combustion method, the factor 0.471 being used. Values for organic matter so obtained are, of course, only approximate and in individual cases may vary widely from the true values. Determinations of pH values were made electrometrically.

DESCRIPTION OF WORK

The samples used in this investigation were selected to represent a wide range of soils which have developed under humid conditions. The profiles varied in their characteristics from the Emmet, a typical podsol, to the Barnes identified as Chernozem. The other profiles selected possessed characteristics intermediate between these extremes. They were mainly from the southern part of the piedmont plateau. This region was represented by three soil series, the Cecil, the Porters, and the Durham, the profiles differing chiefly in degree of maturity. The Cecil profile is fully developed, the Porters somewhat less mature, while the Durham profile is immature. These three profiles have developed from gneiss.

A second Cecil profile derived from mica schist was included in this group to bring out possible differences in the composition of colloidal material that may have been induced by a different kind of parent material. A profile of the Houston series, a Rendzina, was also chosen for examination.

The soil samples were selected especially for this and similar investigations by W. E. Hearn and Mark Baldwin of the Division of Soil Survey. It was especially important for this work that the

soils studied should be developed in place from homogeneous parent material. So far as could be judged by color, texture, and general field appearance these profiles were normally developed and had not recently been disturbed.

A description of the profile samples is given in Table 1. Although certain horizons only of each profile were studied, descriptions of the complete profile are given.

TABLE 1.—Description of the soil profile samples

Profile No.	Soil type	Depth in inches	Horizon	Description	Parent material	Location
1	Emmet fine sandy loam.	0 - 1	A ₀	Litter and sandy humus soil	Glacial drift.	Menominee County, Mich.
		1 - 4	A ₁	Whitish-gray fine loamy sand		
		4 - 12	B ₁	Yellowish, mellow, friable, light fine sandy loam.		
		12 - 24	B ₂	Mottled gray, yellow, and brown, porous, brittle, slightly cemented clayey sand.		
		24 - 33	B ₃	Brown, porous, brittle, gritty, light sandy clay.		
		33 - 48	C ₁	Pale, purplish-gray light drift.		
2	Cecil clay	48 - 60	C ₂	Pinkish-gray, gravelly, light sandy clay drift.	Gneiss.	Rutherford County, N. C.
		0 - 5	A	Reddish-brown clay loam.		
		5 - 36	B ₁	Red, stiff, brittle clay		
		36 - 72	B ₂	Red, friable, micaceous clay		
		72 - 96	C ₁	Brownish-red, friable, partly decomposed rock.		
		96 - 112	C ₂	Gray, soft, disintegrated rock		
3	Cecil sandy clay loam.	112+	C ₃	Soft rock	Mica schist.	De Kalb County, Ga.
		0 - 1½	A ₀	Reddish-brown loamy fine sand		
		1½ - 4	A ₁	do.		
		5 - 8	A ₂	do.		
		9 - 16	B ₁	Red, friable fine sandy clay		
		17 - 22	B ₂	do.		
4	Porters loam.	23 - 62	B ₃	Red, heavy clay	Gneiss.	Rutherford County, N. C.
		63 - 85	C ₁	Red, decomposed rock		
		86 - 108	C ₂	Yellowish-red, partly decomposed rock.		
		120 - 168	C ₃	Yellow, partly decomposed rock		
		180 - 198	C ₄	Grayish-brown disintegrated rock		
		96+		Bedrock		
5	Durham sandy loam.	0 - 4	A	Brown loam	Granite.	De Kalb County, Ga.
		4 - 14	B ₁	Reddish-brown clay loam		
		14 - 48	B ₂	Reddish-brown, friable clay		
		48 - 60	C ₁	Reddish-yellow, partly decomposed rock.		
		60 - 72	C ₂	Gray, disintegrated rock		
		72 - 96	C ₃	Light gray, partly disintegrated rock		
6	Houston clay.	96+		Bedrock	Marl.	Dallas County, Ala.
		0 - 7	A	Light-gray, loose sandy loam		
		19 - 36	B ₁	Light, mottled, yellowish-brown sandy clay loam.		
		37 - 50	B ₂	do.		
		51 - 73	C ₁	Mottled yellowish-red and yellow and gray partly decomposed rock.		
		74 - 90	C ₂	Light-gray and yellow partly decomposed rock.		
7	Barnes silt loam.	91 - 102	C ₃	Very light gray, slightly decomposed rock.	Glacial drift.	Brown County, S. D.
		130 - 110	C ₄	do.		
		110+		Bedrock		
		0 - 4	A	Brown silty clay loam		
		5 - 36	B	Mottled gray, red, yellow, and yellowish-brown, heavy plastic silty clay.		
		37 - 68+	C	Grayish-yellow, heavy, plastic silty clay.		
7	Barnes silt loam.	0 - 2½	A ₀	Black silt loam	Glacial drift.	Brown County, S. D.
		2½ - 7	A ₁	do.		
		8 - 11	A ₂	Dark-brown silt loam		
		11 - 14	B ₁	Light-brown silt loam		
		14 - 48	B ₂	Yellow silt loam		
		28 - 40	B ₃	do.		
7	Barnes silt loam.	44 - 56	C ₁	Brown, yellow, and gray mottled drift		
		60 - 78	C ₂	do.		

CHEMICAL COMPOSITION OF SOILS AND ISOLATED COLLOIDS

The chemical composition of the soils from which the colloidal materials were removed are shown in Table 2. The percentages of colloid isolated and the pH value of each horizon are also shown in the table. In the case of two profiles, Nos. 4 and 5, the composition of the fresh parent rocks is given.

The analyses of the soils indicate in most cases marked accumulation of sesquioxides in the B horizons of all profiles except Nos. 6 and 7, with corresponding reduction in the percentages of silica. These high percentages of iron and alumina are accompanied by increases in percentage of combined water, indicating, of course, concentration of the products of decomposition. The percentages of isolated colloid (column 5) parallel in general the increases in sesquioxides and combined water with certain exceptions. For instance, the quantities of iron, alumina, and combined water are as high in the lower parts of the C horizon of profile No. 3 as they are in the B horizon, although the quantity of colloid removed from these lower horizons is only one-sixth to one-seventh of that removed from the B horizon. This might indicate that relatively little colloid in these lower horizons was removed or that the colloid was unusually high in sesquioxides and combined water. The most probable explanation, however, is that the mineral portion of the C horizon of this profile is composed almost entirely of highly altered muscovite and biotite which, as shown by a previous paper (2), contain within the mica particle varying amounts of material higher in alumina and water than the fresh mica.

A similar high proportion of alumina and water is shown by the sample from horizon C₄ of profile No. 5 in which the percentages of alumina and water are wholly out of proportion to the quantity of colloid isolated from this horizon. In view of the presence of material in mica particles having substantially the composition of colloidal soil material, it is possible that the feldspar particles, of which the horizon in question is chiefly composed, contain somewhat large amounts of material approaching soil colloidal material in composition.

That a degree of weathering has taken place in the transition of the fresh granite of profile No. 5 to the partially decomposed material of the C₄ horizon that is out of proportion to the quantity of colloid removed is also indicated by the low pH value of this horizon, 5.47, and by the fact that at least 38 per cent of the silica originally present in the fresh rock has been lost. In fact, the relatively low pH values of the partially decomposed parent rock in profiles Nos. 2, 3, and 4, which are 4.83, 4.90, and 5.47, respectively, indicate more extensive decomposition than the appearance of the material or the quantity of colloid would indicate. Extensive alteration of the soda feldspars in particular is indicated by the marked reductions in soda which have occurred in the breaking down of the parent rock in profiles Nos. 2, 4, and 5.

The compositions of the isolated colloids are shown in Table 3.

TABLE 2.—Percentage composition and pH values of soils from which colloids (expressed in per cent) were isolated

Profile No.	Soil type and location	Depth in inches	Horizon	Quantity of colloid isolated	pH	SiO ₂	TiO ₂	Fe ₂ O ₃	Al ₂ O ₃	MnO	CaO	MgO	K ₂ O	Na ₂ O	P ₂ O ₅	Ignition	Organic matter	Combined water	CO ₂ from carbonates
a 1	{ Emmet fine sandy loam, Michigan.	1 - 4	A ₁	0.7	5.80	86.18	0.49	1.79	5.57	0.09	0.54	0.18	2.01	0.74	0.05	1.32	1.12	0.20	0
		24 - 33	B ₂	14.1	6.53	70.46	.43	5.05	9.28	.07	1.86	1.65	2.04	.72	.09	2.00	.41	1.59	0
		48 - 60	C ₂	3.0	8.60	71.94	.23	1.38	4.86	.00	0.54	3.90	2.04	.44	.03	8.10	.00	1.43	6.67
2	{ Cecil clay loam, North Carolina.	0 - 5	A	21.5	4.80	64.64	1.81	11.06	15.36	.07	1.38	.73	2.62	.38	.09	11.59	3.80	5.54	0
		5 - 36	B ₁	59.7	5.13	46.00	1.50	11.06	27.26	.05	.18	.67	1.40	.64	.10	0.34	3.80	10.70	0
		72 - 96	C ₁	27.0	5.17	57.78	1.50	9.92	27.40	.15	.64	2.28	3.14	.68	.08	8.68	.43	8.25	0
3	{ Cecil sandy clay loam, Georgia.	112+	C ₃	3.9	4.83	56.72	.80	4.43	22.12	.10	2.54	1.48	5.16	2.94	1.21	2.45	.08	3.00	0
		17 - 22	A ₁	5.4	4.77	64.48	.80	7.11	18.54	.02	.00	.22	1.16	.28	.26	2.45	.95	1.70	0
		180 - 198	C ₂	37.3	5.13	62.73	.71	7.40	19.26	.10	.08	1.22	1.00	.36	.29	7.67	.33	6.48	0
4	{ Porters loam, North Carolina.	0 - 48	C ₄	8.2	4.68	61.08	.74	6.82	19.26	.16	.08	2.15	2.41	.62	.10	6.82	.08	7.52	0
		15 - 48	A	11.0	4.68	73.00	.53	9.24	11.67	.03	.84	.84	4.28	1.28	.06	6.24	.39	2.27	0
		72 - 96	C ₁	36.9	5.05	60.34	.68	4.92	21.07	.04	.24	.80	3.14	.60	.08	8.02	.46	7.56	0
5	{ Durham sandy loam, Georgia.	0 - 7	Bedrock.	3.2	7.65	71.50	.50	3.52	14.21	.05	.28	.74	5.30	.84	.08	4.22	.09	4.13	0
		51 - 73	A	15.7	5.52	70.33	.15	2.72	14.21	.05	1.84	.61	4.52	2.84	.42	.44	.06	.28	0
		91 - 102	C ₁	1.7	4.67	68.28	.14	1.02	19.04	.02	.33	.06	5.15	1.01	.26	2.28	.86	1.42	0
6	{ Houston clay, Alabama.	0 - 4	C ₄	2.8	5.10	70.51	.08	1.02	17.46	.02	.24	.18	4.04	1.40	.15	4.01	.27	4.64	0
		5 - 36	A	52.1	4.90	63.81	.12	9.1	15.24	.03	.40	.34	4.28	.46	.25	3.85	.19	3.66	0
		37 - 68	C	65.1	4.73	61.14	.07	7.15	16.25	.09	1.04	1.00	1.16	.30	.09	.61	.06	.56	0
a 7	{ Barnes silt loam, South Dakota.	0 - 21 ^a	B ₂	22.8	6.60	58.44	.92	7.82	20.54	.03	1.66	1.24	1.12	.30	.36	7.76	.62	7.57	0
		14 - 48	A ₀	20.2	7.00	70.74	.60	3.36	9.55	.14	1.66	.96	2.11	1.80	.26	10.16	.73	2.86	0
		60 - 78	C ₂	11.3	8.45	68.64	.44	3.95	11.73	.11	1.31	1.27	1.93	1.13	.13	4.49	1.08	2.81	5.87

^a The writer is indebted to G. Edgington for the complete analyses of the samples from horizons A and B, profile No. 1, and for determinations of P₂O₅, MnO, K₂O, Na₂O, and CO₂ as carbonates in the samples from profiles Nos. 1 and 7.

^b The small amounts of organic matter in these horizons could not be detected by the method used in the presence of large amounts of carbonate.

^c Trace.

^d Not determined.

TABLE 3.—Percentage composition and pH values of colloidal materials (expressed in per cent) isolated from the horizons of various soil profiles

Profile No.	Soil type and location	Depth in inches	Horizon	Quantity of colloid isolated	pH of soil	SiO ₂	TiO ₂	Fe ₂ O ₃	Al ₂ O ₃	MnO	CaO	MgO	K ₂ O	Na ₂ O	I ₂ O ₅	Ignition	Organic matter	Combined water	CO ₂ as carbonates
1	Emmet fine sandy loam, Michigan.	1-4 24-30 48-60	A ₁ B ₁ C ₂	0.7 14.1 3.0	5.80 6.53 8.60	41.10 42.43 41.66	0.90 1.76 1.09	6.46 13.31 10.66	19.67 21.02 13.21	0.44 1.05 1.09	1.54 1.05 6.42	1.92 3.03 6.52	2.00 2.74 3.46	0.26 0.70 3.87	0.59 0.22 0.92	25.81 15.16 15.32	15.18 2.35 2.38	10.63 1.01 1.02	0 7.02 0
2	Cecil clay loam, North Carolina.	0-5 5-26 72-112+	A ₁ B ₁ C ₁	21.5 59.7 27.0	4.75 5.13 5.37	31.69 32.44 28.20	0.90 1.12 1.10	10.81 13.73 10.03	29.61 34.71 38.21	1.10 1.05 1.15	6.42 5.26 5.24	6.52 5.53 7.70	3.46 3.63 1.33	0.73 0.28 0.32	0.25 0.25 0.44	24.65 16.33 24.30	10.86 2.54 3.74	12.79 13.70 16.06	0 0 0
3	Cecil sandy clay loam, Georgia.	17-22 86-108 180-193	A ₁ B ₁ C ₁	37.5 5.3 6.5	4.87 5.13 5.13	31.30 34.12 36.20	1.01 1.16 1.46	11.60 14.18 7.34	37.64 31.15 32.08	0.7 1.16 1.46	1.54 1.71 1.71	1.92 1.28 1.73	2.00 1.94 0.73	0.58 0.54 0.57	0.24 0.35 0.35	18.12 15.64 17.41	6.33 1.92 5.50	11.79 13.72 12.41	0 0 0
4	Porters loam, North Carolina.	0-4 14-49 72-96	A ₁ B ₂ C ₃	11.0 36.6 5.2	4.68 5.05 5.10	29.58 30.74 30.74	0.84 0.64 0.22	8.45 8.03 2.13	28.62 36.39 38.41	0.04 0.12 0.12	0.36 0.20 0.24	1.46 0.58 0.30	1.02 1.06 0.52	0.40 0.74 0.38	0.02 0.47 0.20	17.41 26.13 18.32	5.00 10.41 2.18	12.77 12.72 14.41	0 0 0
5	Durham sandy loam, Georgia.	51-73 91-102 103-110	A ₁ C ₁ C ₄	15.7 2.9 1.7	4.67 5.22 5.10	38.06 25.92 22.41	0.40 0.26 0.67	5.68 4.11 2.45	38.14 33.33 43.26	0.03 0.03 (^a)	0.46 0.52 0.52	0.64 0.70 0.50	1.06 0.93 1.18	0.30 0.38 0.60	0.19 0.43 1.28	16.37 25.66 29.56	2.36 6.83 11.26	14.01 18.83 18.30	0 0 0
6	Houston clay, Alabama.	0-4 5-36 37-98	A ₁ B ₁ C	52.1 65.1 64.7	4.90 4.73 5.60	44.60 45.39 47.10	0.57 0.58 0.70	8.68 9.02 8.72	25.69 27.03 25.84	0.02 0.03 0.25	1.61 1.47 1.83	1.78 1.86 2.22	1.14 1.01 1.36	0.67 0.97 0.99	0.25 0.37 0.50	15.07 13.71 12.87	5.66 3.95 3.22	9.41 9.76 10.88	0 0 0
7	Barnes silt loam, South Dakota.	14-48 60-78	A ₀ B ₂ C ₂	22.8 20.15 11.31	6.40 7.22 8.45	40.98 46.84 51.74	0.50 0.66 0.64	9.08 11.74 11.74	18.35 18.35 17.36	0.17 0.17 0.13	1.40 1.70 1.70	3.02 3.80 3.80	1.36 1.98 1.98	0.11 0.15 0.15	0.29 0.18 0.18	16.83 10.32 10.32	17.76 2.33 2.33	9.06 7.99 7.99	0 1.27 1.27

^a Determinations of MnO, K₂O, Na₂O, P₂O₅, and CO₂ as carbonates in colloid samples from profiles Nos. 1 and 7 by G. Edgington.

^b Trace.

The analytical data given in Table 3 show that in certain profiles the colloids are fairly constant in composition in all horizons but that in other profiles they vary rather widely. For instance, the colloids in the Houston profile (No. 6) are essentially similar in composition throughout the profile. In the Durham profile (No. 5), however, marked variations are to be noted in silica, alumina, phosphoric acid, and in combined water. In nearly all profiles the iron content varies considerably, the highest concentration occurring usually in the A and B horizons. The percentage of the bases shows little variation with depth except in the colloid from those profiles which have developed from glacial material, Nos. 1 and 7. In the colloid from all other profiles the bases are relatively low at all depths. Organic matter instead of being very low in the colloid of the lower horizons, as might be expected, is actually present in considerable quantity in the colloids from the C horizons. The quantities of colloid, however, are very small in the deepest parts of the profile, and small amounts of organic matter penetrating to these depths make up a large proportion of the total colloid. This concentration of organic matter in certain horizons tends to obscure the extent of variation or constancy of the colloid and also the magnitudes of the major constituents. These effects are avoided by recalculating the results to the inorganic basis. The effect of CO_2 as carbonates is also eliminated. The data are given in Table 4.

TABLE 4.—Major constituents of soil colloidal materials, the results being expressed as percentages of the inorganic material minus CO_2 as carbonates

Profile No.	Soil	Depth in inches	Horizon	SiO_2	Fe_2O_3	Al_2O_3	Combined water	Molecular ratio $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$
1	Emmet	1 - 4	A ₁	48.48	7.59	23.19	12.46	2.94
		24 - 33	B ₁	43.91	13.74	21.70	11.36	2.45
		48 - 60	C ₂	47.66	13.40	15.18	11.36	3.40
2	Cecil	0 - 5	A	35.55	12.12	33.22	15.47	1.47
		5 - 36	B ₁	33.28	14.09	35.61	14.15	1.27
		72 - 96	C ₁	29.30	10.42	39.70	16.67	1.08
		112+	C ₃	29.31	4.88	41.37	16.08	1.11
		112 - 4	A	33.41	12.45	35.91	12.59	1.30
3	do	17 - 22	B	30.48	14.33	37.32	13.99	1.11
		86 - 108	C ₁	35.76	14.86	32.65	12.33	1.44
		180 - 198	C ₃	41.36	7.73	33.27	13.06	1.82
		0 - 4	A	35.38	10.11	34.23	15.21	1.48
4	Porters	14 - 48	B ₂	37.14	8.21	37.20	14.89	1.49
		72 - 96	C ₃	41.35	2.22	39.97	15.00	1.70
		0 - 7	A	39.64	6.06	38.21	14.42	1.68
		51 - 73	C ₁	38.98	4.21	40.09	14.38	1.55
5	Durham	91 - 102	C ₃	27.82	2.63	46.43	20.21	.99
		103 - 110	C ₄	25.25	2.62	46.97	20.62	.88
		0 - 4	A	47.28	9.20	27.23	9.97	2.43
6	Houston	5 - 36	B	47.25	9.39	28.14	10.16	2.35
		37 - 68	C	48.66	9.01	26.70	9.97	2.55
		0 - 21½	A ₂	49.96	11.04	18.18	13.23	3.30
7	Barnes	14 - 48	B ₂	50.78	12.73	19.90	9.82	3.09
		60 - 78	C ₂	57.02	12.94	19.13	8.80	3.55

Both silica and alumina vary in a regular manner in all profiles in which the colloid is not practically constant in composition. This variation, however, is not always in the same direction. In the Durham profile the percentage of silica decreases markedly with depth, whereas alumina increases. In the Porters profile, however, both silica and alumina increase from the surface downward.

The variation of silica in relation to the combined values for alumina and iron is shown by comparing the silica-sesquioxide ratios of the colloids in the various horizons. In the case of three profiles, Nos. 1, 3, and 4, the ratio decreases from the parent material toward the surface, following the order which might reasonably have been predicted. The older colloid in the surface horizons having been subjected to extensive leaching would be expected to have lost silica, thereby narrowing the ratio. In two other profiles, Nos. 2 and 5, the silica-sesquioxide ratio follows the reverse order, being smallest in the colloid isolated from the parent material and increasing regularly toward the surface. The lowest ratios are associated with unusually high values for combined water, namely, about 20 per cent.

FRACTIONATION OF THE COLLOIDAL MATERIAL

The unusually high percentages of alumina and combined water and the low percentages of silica in the colloid samples from the C_1 and C_3 horizons of profile No. 2 and from the C_3 and C_4 horizons of profile No. 5 (Table 4) indicated the presence in these colloidal materials of free hydrated alumina. These samples were obtained from horizons of decomposing rock whose compositions (Table 2) indicated extensive mineral alteration although relatively slight amounts of colloid were isolated. It would seem to follow that the bulk of the colloid present in these horizons forms a part of the partially altered mineral particles, the colloid existing presumably on the surfaces. This colloidal material, it was thought, might consist of hydrated alumina from which the siliceous colloid in the form of aggregates might be separated by some mechanical means.

Previous attempts by Robinson and Holmes (9) to fractionate soil colloidal material gave negative results. They concluded that separate colloidal particles differing in composition probably did not exist in the colloidal mixture. This conclusion, however, was reached from a study of colloids from the A and B horizons only and may not hold for colloid in the lower parts of the C horizon, where the colloid is perhaps not so intimately mixed.

The soil samples selected for fractionation of colloidal material were those whose colloids gave indications of containing free alumina. These samples were from the C_1 and C_3 horizons of profile No. 2 and from the C_3 and C_4 horizons of profile No. 5. (Table 4.) In addition to these samples two others were selected, the C_1 horizon of profile No. 5 and the C_3 horizon of profile No. 3. Two colloid fractions from each of the six samples were prepared. The first fraction was made as follows: A 10 to 20 gm. sample of soil material was moistened with water and the mass worked to a somewhat plastic consistency by rubbing the particles between the fingers. A small quantity of water made alkaline with ammonia was then added and after settling for a few minutes the suspension was decanted into a 500 c. c. glass cylinder. The settled portion was rubbed between the fingers as before, and the process continued until the cylinder was filled. The suspension was now allowed to settle until microscopic examination showed that the suspension to a certain depth was practically free of particles more than 1 micron in diameter. The colloidal solution was then drawn off to this depth and evaporated to dryness. The second fraction was prepared in a similar manner except that the silty mate-

rial obtained by removal of the greater part of the colloid (explained under Methods) was employed instead of the soil material. In preparing this second fraction about 50 gms. of material were dispersed in 3 liters of water (alkaline to phenolphthalein) contained in a beaker and the colloidal solution drawn off when nearly all particles more than 1 micron in diameter had settled. The quantities of colloid obtained in all cases were very small, averaging not more than 0.5 gm. This was in consequence of the fact that the soil material of the C horizons contained very little colloid and of the fact that this colloid in the case of the silty materials had previously been largely removed. This scarcity of material necessitated the use of samples for analysis much smaller than those customarily taken, with the result that determinations of the constituents present in small amounts were affected by unusually large errors of analysis. The values given for the mono and divalent bases in Table 5 are, therefore, to be considered as only approximate and may in certain cases depart widely from the true values.

In Table 5 are shown: (1) The composition of fractions of colloidal material obtained by an incomplete dispersion of soil material in water, and (2) the composition of colloidal material obtained by dispersing the corresponding silt fractions from which the greater part of the colloid had previously been removed. The composition of these colloid fractions is compared in the table with the composition of the gross colloidal materials previously given in Tables 3 and 4. This material was obtained by alternate dispersion of the soil material and centrifuging of the suspension until nearly all the colloidal material was removed. There are available then for comparison with this gross colloid the composition of fractions differing in thoroughness with which the soil material was dispersed.

It will be seen from Table 5 that in the case of the horizons below the C₂ horizon the two colloid fractions differ materially in composition from the gross colloidal material. The fractions with a few exceptions contain less silica and more combined water than the corresponding gross colloids. The values for combined water in the case of two fractions are especially noteworthy, being 35.33 and 37.39 per cent. Alumina is seen to be highly variable. It is usually present in greatest amount in the colloid fraction from the soil material and least in the colloid fraction from the silt material. The composition of the bases is generally irregular and shows rather wide variations in the colloids from the same horizon and from different horizons of the same profile. The percentages of calcium oxide, however, seem to follow a rather definite order, being greatest in the colloid from the silt material, less in the colloid fraction from the soil material, and least in the gross colloidal material. The lowest values for magnesia appear usually in the colloid fraction from the soil material. Marked irregularities are shown by the alkalis, but the values given may not in all cases be significant. Phosphoric acid is in most cases extremely high in the colloid fraction from the silt material. It is present in least amount in the gross colloid. The extraordinarily high values for phosphoric acid shown by the two colloid fractions from the C₃ horizon of profile No. 2, 12 and 19 per cent, would indicate the presence of some phosphate of aluminum in these colloids. Organic matter is invariably highest in the colloid fractions from the silt material and least in the fractions from the soil material. These facts indicate that in these soils the difficultly dispersible colloid is largely organic in nature.

TABLE 5.—Percentage composition of soil colloidal fractions compared with compositions of gross colloidal materials

Pro- file No.	Soil	Depth in inches	Hor- izon	Nature of material	SiO ₂	TiO ₂	Fe ₂ O ₃	Al ₂ O ₃	MnO	CaO	MgO	K ₂ O	Na ₂ O	P ₂ O ₅	Igni- tion	Organic matter	Comb- ined water
5	Durham	51-73	C ₁	Colloid fraction from silt material	29.44	(*)	4.27	30.00	(*)	1.70	0.50	2.00	1.94	1.82	29.09	(*)	(*)
				Gross colloidal material	38.19	0.35	5.48	36.94	(*)	.42	.55	.76	.29	.37	15.40	1.57	13.83
		91-102	C ₃	Colloid fraction from silt material	38.06	.40	4.11	39.14	(*)	.46	.64	1.06	.30	.10	10.37	2.36	14.01
				Gross colloidal material	33.35	0	3.31	37.85	(*)	3.51	.80	2.34	1.17	2.61	35.84	12.45	23.39
		108-110	C ₄	Colloid fraction from silt material	16.91	0	3.03	46.45	(*)	.92	.37	1.52	2.17	1.38	26.20	1.34	24.86
				Gross colloidal material	25.92	.26	2.45	43.26	(*)	.52	.70	.93	.38	.43	25.66	6.83	18.83
				Colloid fraction from silt material	11.50	(*)	3.65	27.32	(*)	.61	.77	.75	.98	3.32	50.76	13.37	37.39
2	Cecil	72-96	C ₁	Colloid fraction from silt material	20.90	(*)	2.15	45.04	(*)	.42	.38	.50	(*)	1.47	27.47	3.27	24.20
				Gross colloidal material	22.41	(*)	2.33	41.68	(*)	.40	.50	.28	1.76	1.86	29.56	11.26	15.30
		112+	C ₃	Colloid fraction from silt material	27.54	1.04	7.60	32.06	(*)	1.00	1.98	.28	1.76	1.86	23.70	8.03	14.77
				Gross colloidal material	28.68	1.12	10.95	39.84	.14	.23	.14	.18	.09	.78	17.37	.86	16.51
		180-198	C ₃	Colloid fraction from silt material	35.20	0	10.03	38.21	.15	1.24	.02	1.07	0	.84	19.82	3.76	16.06
3	do	51-73	C ₁	Colloid fraction from silt material	11.08	(*)	1.69	37.88	(*)	.60	.42	.60	.55	11.89	24.82	2.51	21.81
				Gross colloidal material	26.47	.46	4.40	37.60	.08	.42	.33	1.43	.24	3.44	24.90	9.85	14.80
		91-102	C ₃	Colloid fraction from silt material	26.42	.31	4.30	37.32	(*)	1.45	1.45	(*)	(*)	.65	42.74	7.41	35.33
				Gross colloidal material	33.70	.57	8.26	38.04	(*)	.45	.90	(*)	(*)	.06	24.88	2.79	21.79
		180-198	C ₃	Colloid fraction from silt material	39.29	.46	7.34	32.06	(*)	.20	1.46	1.02	.40	.33	17.41	5.00	12.41
				Gross colloidal material	39.29												

* Trace.

† Not determined.

* Analysis by G. Edgington.

In order to make a more careful study of the proportions of the major constituents the values for these constituents shown in Table 5 were corrected for the organic matter in the samples. These values are shown in Table 6.

TABLE 6.—Major constituents (percentage) of soil colloid fractions and of gross colloidal material

[Results on inorganic basis]

Profile No.	Soil	Depth in inches	Horizon	Nature of material	SiO ₂	Fe ₂ O ₃	Al ₂ O ₃	P ₂ O ₅	Combined water	Molecular ratio	
										SiO ₂	Al ₂ O ₃ +Fe ₂ O ₃
5	Durham	51-73	C	Colloid fraction from silt material.	(*)	(*)	(*)	(*)	(*)		1.50
				Colloid fraction from soil material.	38.80	5.56	37.53	0.37	14.05		1.60
				Gross colloidal material.	38.98	4.21	40.19	.19	14.35		1.55
		91-102	C	Colloid fraction from silt material.	15.24	3.78	43.23	2.98	26.71		.57
				Colloid fraction from soil material.	17.04	3.07	46.43	1.40	25.20		.56
				Gross colloidal material.	27.82	2.63	50.12	.46	20.21		.99
		103-110	C ₄	Colloid fraction from silt material.	13.16	4.21	31.53	3.83	43.16		.66
				Colloid fraction from soil material.	21.61	2.22	46.56	1.52	25.02		.77
				Gross colloidal material.	25.25	2.62	46.97	1.44	20.62		.88
		72-96	C ₁	Colloid fraction from silt material.	30.24	8.34	35.21	2.04	16.22		1.08
				Colloid fraction from soil material.	28.95	10.29	39.23	.86	16.52		.97
				Gross colloidal material.	29.30	10.42	39.70	.87	16.67		1.27
2	Cecil	112+	C ₃	Colloid fraction from silt material.	12.12	2.60	33.85	20.90	26.93		.58
				Colloid fraction from soil material.	20.48	2.04	38.85	12.20	22.37		.87
				Gross colloidal material.	29.31	4.88	41.37	3.82	16.08		1.11
		180-198	C ₃	Colloid fraction from silt material.	27.46	4.26	22.92	.70	38.16		1.82
				Colloid fraction from soil material.	34.76	8.50	28.84	.78	22.42		1.10
				Gross colloidal material.	41.36	7.73	33.77	.37	13.06		1.82

* Not calculated.

The effect of calculating the data of Table 5 to the inorganic basis has been to increase markedly the values of alumina and combined water in those samples in which organic matter was abundant. The values for alumina on the inorganic basis show a regularity not observed in Table 5. The highest values for alumina are found in the gross colloidal material and the least in the fraction from the soil material. It will be observed, however, that low values for alumina are associated with high values for combined water. If the values for alumina in the three colloids of each horizon are calculated to the same water content, the differences in alumina largely disappear.

One of the most significant features of the data of Table 6 is the marked differences in the silica-sesquioxide ratio shown by the groups of three colloids. In the case of the colloids from the C₃ and C₄ horizons of profile No. 5, the ratios of silica to alumina plus iron in the colloid fractions are much lower than the corresponding values in the gross colloidal material. The low ratios observed in the case

of the fractions from the C_3 horizon of profile No. 2 are in consequence of the fact that a considerable part of the alumina present is combined with phosphoric acid and is, therefore, not properly a part of the colloid complex. It is evident, of course, that none of the fractions are composed entirely of material of definite composition, since a sharp separation of definite kinds of colloid was not possible. It may be assumed from the fact that the silica-sesquioxide ratio apparently may diminish to zero that one component of the mixture is free hydrated alumina.

DISCUSSION

The colloidal material of certain of the horizons studied appears to be a mixture of hydrated alumina associated chiefly with the mineral portion of the soil and of siliceous colloid occurring chiefly as colloidal aggregates. Incomplete dispersion of the soil material removed chiefly the aluminous colloid, since the rubbing of the material was insufficient to disperse the siliceous aggregates. In the course of the more thorough dispersion required to remove the bulk of the colloidal material, these aggregates were broken down as is shown by the relatively high percentages of silica in the gross colloidal material. However, after removal of practically all of the colloid present as aggregates, succeeding fractions of colloid were aluminous.

The presence of free alumina in the lower horizons of the soil profile does not mean necessarily that the alumina was produced in those horizons. The downward movement of sesquioxides from the A to the B horizon is, of course, a characteristic feature of podsollic soils. In fact, it has been pointed out that iron may be removed from the soil profile and accumulated elsewhere as bog iron ore. That alumina may penetrate to great depths in the soil profile, however, has not been recognized. The mobility of alumina in the soil depends, of course, on the stability of alumina sols under the conditions prevailing in the soil. Among these conditions may be enumerated the following: Reaction of the soil, concentrations of anions and cations, and the relative concentrations of sols of alumina, silica, iron, and organic matter. Since these factors are closely interrelated it would be very difficult to evaluate them in the case of any one soil. It is interesting, however, to consider these factors in a general way.

Magistad (6) concluded that alumina in amounts greater than 3 parts per million can exist in the soil in solution only when the reaction is more acid than pH 4.7 or less acid than pH 8. From pH 5.4 to pH 7 the amount of alumina in solution is usually less than a part per million. However, Joffe (5) points out that these values are valid in the presence of the sulphate anion only. In fact, according to Joffe the sol state of alumina can not exist in the presence of the sulphate ion. In the presence of the chloride ion the sol exists from pH 4.3 to pH 5; at the latter value most of the alumina is in the gel state. The nitrate ion influences the state of aggregation in about the same manner as the chloride ion.

In regard to the effect of organic-matter sols on the stability of alumina sols Aarnio's work (1) may be cited. Aarnio found that whereas iron oxide sols were stabilized by organic matter if the concentration of organic matter was three times that of the iron oxide, alumina sols required thirty times more humus than alumina to effect

stabilization. He concluded from this relationship that aluminum precipitates under all circumstances since such concentrated humus sols do not usually exist in nature.

The presence of considerable quantities of organic matter in the colloid fractions obtained by dispersing the silt material suggests the possibility that alumina may have penetrated to the lower horizons under the stabilizing effect of soluble organic matter. From Aarnio's work, however, it would seem that conditions favored the accumulation of ferric oxide in these lower horizons rather than alumina. The composition of the colloid fractions, however, gave no indication that ferric oxide had accumulated.

The recent work of Mattson (7) on the mutual flocculation of alumina and silica solutions is of interest in considering the possibility that alumina may have penetrated downward under the stabilizing action of silica. Mattson (7, *p.* 302) concluded that "the alumina-silica system forms isoelectric precipitates in which the proportion of silica decreases with an increase in pH, approaching zero at pH 7 at which point the alumina is itself isoelectric." According to Mattson alumina and silica in the molecular ratio of 1 to 1 would be flocculated at pH 6.6, and a mixture of silica to alumina in the ratio of 6 to 1 would flocculate at pH 5. These relationships are sufficient to render the transport of alumina under the protective action of silica highly improbable in the soils under study.

Although no definite conclusion can be reached as to the mobility of alumina in these particular soils, it has been seen that soil conditions are in general unfavorable to the downward movement of alumina below the B horizon. In the absence of evidence that alumina has moved downward in these soils it is important to consider the possibility that the alumina may have been formed in the horizons in which it occurs.

Evidence was offered earlier in this paper which indicated that mineral particles in certain of the horizons were highly altered although relatively little colloid could be isolated from the horizons in question. This condition was accounted for by assuming that the greater part of the colloidal material present in these horizons was contained on the surfaces of certain mineral particles, relatively little being present in the form of aggregates. It has been shown that colloid fractions prepared by further dispersion of silt material from which the greater part of the colloid had previously been removed contained a higher proportion of alumina to silica than was present in the gross colloidal material. It is reasonable to conclude, therefore, that this excess alumina was present on the surfaces of mineral particles rather than as colloidal aggregates. It might be assumed, of course, that the alumina was derived from difficultly dispersible aggregates consisting chiefly of colloidal alumina. This explanation, however, is rendered improbable by the fact that the colloid fractions prepared by partial dispersion of the soil material were similar to those prepared by dispersing the silt fractions, the differences between the two fractions being chiefly in degree of hydration. Furthermore, the large amounts of combined water contained in several of the colloid fractions indicate the relatively recent origin of the material.

The alumina present in the horizons investigated may be regarded as derived from some intermediate alteration product of silicate min-

erals. Kaolinite is perhaps too resistant to alteration to have produced the alumina, but halloysite, its amorphous equivalent, may have been the intermediate alteration product from which the alumina was derived. The association of halloysite with bauxite and the alteration of plagioclase feldspars to bauxite are, perhaps, significant in this connection.

The conclusions reached in regard to the origin of colloidal alumina in these soils are wholly tentative, further inquiry having been terminated by the author's transfer to another bureau. The data are published at this time by reason of their unique nature. Conclusions suggested by the data should be tested by further investigation.

SUMMARY

This investigation deals with the composition of colloidal material in the horizons of the soil profile. Colloidal material was isolated quantitatively from seven soil profiles representing a wide range of composition and profile characteristics. It was found that in some profiles the colloidal materials were fairly constant in composition; in other profiles the colloids varied rather widely. The constituents in which variations were most general were silica, alumina, iron, organic matter, and combined water. Except in the case of profiles developed from glacial material the percentages of the bases showed little variation with depth and were low in all horizons. Silica and alumina varied regularly in all profiles in which the colloids were not practically constant in composition. These variations, however, were not always in the same direction.

The molecular ratio of silica to alumina plus iron showed in the case of colloids from several profiles a regular decrease with depth, indicating that extent of weathering was not the chief factor tending to reduce this ratio. The relatively low silica-sesquioxide ratios were attributed to the presence of free hydrated alumina in the colloidal material of the lower horizons. Further evidence of the presence of free alumina was obtained by fractionating the colloid showing a low silica-sesquioxide ratio. The colloidal fractions were found to contain a higher proportion of alumina to silica and more combined water than the gross colloidal materials. The presence of free alumina in the colloid of the lower horizons was accounted for on the assumption that colloidal material existing on the surfaces of altering mineral particles contains free alumina.

The relatively high percentages of alumina and combined water in disintegrated but apparently undecomposed parent rock and the low pH values of this material indicate that some of the minerals composing this material have undergone extensive alteration.

LITERATURE CITED

- (1) AARNIO, B.
1928. CHEMICAL AND PHYSICAL PROPERTIES OF FINNISH SOILS. First Internatl. Congr. Soil Sci. Comm. 5, Proc. and Papers 4: 507-523, illus.
- (2) DENISON, I. A., FRY, W. H., and GILE, P. L.
1929. ALTERATION OF MUSCOVITE AND BIOTITE IN THE SOIL. U. S. Dept. Agr. Tech. Bul. 128, 32 p.
- (3) HILLEBRAND, W. F.
1919. THE ANALYSIS OF SILICATE AND CARBONATE ROCKS. U. S. Geol. Survey Bul. 700, 285 p., illus.

-
- (4) HOLMES, R. S.
1928. VARIATIONS OF THE COLLOIDAL MATERIAL IN TYPICAL AREAS OF THE LEONARDTOWN SILT LOAM SOIL. *Jour. Agr. Research* 36: 459-470.
- (5) JOFFE, J. S., and McLEAN, H. C.
1928. PROBABLE INFLUENCE OF ANIONS ON ALUMINUM SOLUBILITY IN SOILS. *First Internatl. Congr. Soil Sci. Comm. 2, Proc. and Papers* 2: 230-255, illus.
- (6) MAGISTAD, O. C.
1925. THE ALUMINUM CONTENT OF THE SOIL SOLUTION AND ITS RELATION TO SOIL REACTION AND PLANT GROWTH. *Soil Sci.* 20: 181-225, illus.
- (7) MATTSON, S.
1928. THE ELECTROKINETIC AND CHEMICAL BEHAVIOR OF THE ALUMINO-SILICATES. *Soil Sci.* 25: 289-311.
- (8) MCCOOL, M. M.
1927. RESULTS OF SOME PHYSICAL AND CHEMICAL STUDIES ON SOIL COLLOIDS. *Jour. Amer. Soc. Agron.* 19: 289-297.
- (9) ROBINSON, W. O., and HOLMES, R. S.
1924. THE CHEMICAL COMPOSITION OF SOIL COLLOIDS. *U. S. Dept. Agr. Bul.* 1311, 42 p.

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A STUDY OF THE COTTON FLEA HOPPER, *PSALLUS SERIATUS* REUT., WITH ESPECIAL REFERENCE TO ITS EFFECT ON COTTON PLANT TISSUES^{1 2}

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INTRODUCTION

The cotton flea hopper, *Psallus seriatus* Reuter, which belongs to the family Miridae, has only recently become important as a cotton pest (5).⁴ The spread through the Southern States of a peculiar form of injury traceable to this insect has been rapid, although the insect itself has been known to be present there for some time. The generalized effect of the attack of the insect on the cotton plant leads one to look for the presence of other factors in addition to simple insect feeding.

During the summer of 1925 an investigation was made of the possibility of the transmission of a plant disease by the cotton flea hopper, and in order to get a definite anatomical basis for future work both with regard to the insect and the injured plant structure the study was made in three parts: (1) A study of the anatomy of the digestive apparatus of the insect; (2) a study of pathological and of healthy plant tissue; and (3) an examination of the fresh material for verification of findings in preserved material and examination of digestive enzymes present.

TECHNIC

The material intended for preservation and later study was collected at Port Lavaca, Tex., and after fixation was preserved in 80 per cent alcohol until used at Columbus, Ohio. Eight different fixing fluids were used. Bouin's, Zenker's, and Petrunkevitch's fixing fluids were found to be the best for insect material. Bouin's fluid and a formalin-acetic acid-alcohol formula by Chamberlain (2) were found to be the best for the plant material. Heidenhain's iron haematoxylin, used by the longer method, and Ehrlich's acid haematoxylin, both counterstained with orange G., were found to be the most satisfactory stains for both plant and insect tissues.

¹ Received for publication July 24, 1929; issued March, 1930.

² This paper was presented in August, 1926, as a dissertation in partial fulfillment of the requirements for the degree of doctor of philosophy in the graduate school of the Ohio State University. The work upon which it is based was begun while the author was in the status of temporary field assistant in the Bureau of Entomology, U. S. Department of Agriculture, and completed in 1926 while he was in the status of collaborator of the Bureau of Entomology and graduate student, Ohio State University.

³ The first experiments were made under the direction of the late Dr. W. D. Hunter, to whom the writer is indebted for many useful suggestions. The writer wishes to thank the members of the faculty of the department of zoology and entomology of Ohio State University, especially Drs. Herbert Osborn, Raymond C. Osburn, and C. H. Kennedy, and Dr. H. C. Sampson, of the department of botany, for valuable assistance and suggestions. Thanks are also due to members of the U. S. Bureau of Entomology, especially to Mr. B. R. Coad and Dr. J. W. Folsom, for making possible the investigation and for the excellent facilities furnished at the Delta laboratory, Tallulah, La., during July, 1926. The writer wishes also to thank Mr. Lawrence Youman, of Columbus, Ohio, for help with the photomicrographs of plant tissue. Most of the microtechnic work in connection with the preparation of slides for study was done by Mrs. R. H. Painter, to whose efforts was due the excellence of the material that was studied. This manuscript was submitted to the Bureau of Entomology for publication Sept. 28, 1926.

⁴ Reference is made by number (italic) to "Literature cited," p. 516.

In studying the gross anatomy of these minute insects the following method was used for dissection and found to be successful if a strong enough illumination was used: The insect which had been fixed and stored was removed from the alcohol and allowed to dry a few minutes on filter paper. It was then placed on its side in a small drop of melted paraffin on the bottom of a watch-glass dissecting dish and completely covered with a thin coat of paraffin. The dish was flooded with cold 50 per cent alcohol and placed under the binocular. Scalpels were prepared by cementing a scrap of safety-razor blade in the end of a glass tube. With these it was possible to remove the exoskeleton together with the paraffin and expose the internal organs. The alcohol was then poured off. A drop of Ehrlich's acid haematoxylin was placed on the insect and allowed to stand for about five minutes and was then washed off. Dissection was then resumed under alcohol. This method does not stain entirely through the insect at one time, and it is often necessary to repeat the staining process. The coloring of the insect, a part at a time, is often of advantage. Organs may be stained in this manner and removed for mounting on slides.

Although a number of other hemipterous forms have been studied with regard to their internal anatomy and histology, relatively little is known about the Miridae in this respect.

Dufour (3), in 1833, figured and described the anatomy of the digestive tract of two European mirids, but the writer has been unable to find a description of the histology of any member of the family.

STRUCTURE AND HISTOLOGY OF THE ALIMENTARY CANAL AND ITS APPENDAGES

The structure of the mouth parts and salivary apparatus of *Psallus seriatus* is, on the whole, similar to that of other Hemiptera. With regard to homologies of parts the writer has followed the terminology⁵ given in a General Textbook of Entomology, by Imms (6), and no attempt has been made to study these parts in regard to their relationship to similar parts in other members of the order. Hence the parts will be only briefly described and any structures peculiar to this insect will be noted. The description below applies to the adult except as otherwise mentioned.

MOUTH PARTS

The labium is 4-segmented and is furnished at the tip, as is usual in the order, with sensory hairs and pits. The proximal segment is quite heavily muscled for the bending of this organ at the time of feeding. This musculature, however, has not been worked out in detail. A groove, which is not entirely closed at any point, runs the length of this rostrum, except at its extreme basal end, and contains the stylets. Above the basal end of these mouth parts are the epipharynx and labrum, which are not easily distinguishable from each other. At the base of these is a groove in which the stylets lie.

The stylets consist of the mandibles and maxillae, all of which are hollow except at the extreme tips, and at their bases possess glands and muscular attachments. In the head each pair of stylets

⁵ A paper by Snodgrass (9), which appeared after this manuscript was submitted for publication, gives a different and probably better interpretation of the parts of the hemipteran head. An explanation of the function of the "retort-shaped organs" described herein is also given in his paper.

is somewhat enlarged and flattened, the mandibles lying above the maxillae. As they pass into the groove of the epipharynx, the mandibles take up a position on each side of the maxillae. The latter possess two grooves on the inner faces which, when opposed to one another, form dorsally the suction canal and ventrally the ejection canal. The former connects with the pharynx, the latter with the salivary duct, opening through the hypopharynx. The stylets themselves are interlocked and grooved (fig. 1, D) and are furnished with ridges which fit into grooves in the ventral plate of the epipharynx where the stylets emerge from the head. At their tips the mandibles are of a heavier structure and are slightly shorter than the maxillae, and, whereas the latter are thin and barbed on their dorsal edge, the former are apparently smooth.

In the attachment of the stylets there are apparently no levers such as are present in some of the other Hemiptera, but each appendage is attached by a pair of muscles, as shown in Figure 1, E and F. In the head the upper and inner pair of stylets are the mandibles. The small dorsal muscle of the mandible attaches to the vertex after passing out just in front of the optic nerve. The ventral muscle passes beneath and to the side of the brain and attaches to the occiput or back wall of the head capsule. (Fig. 1, E.) On the maxillae the muscles are larger; one passes anteriorly, attaching to the maxillary sclerite near its junction with the labrum; the other extends posteriorly, passing beneath and behind the optic nerve and attaching to the occiput just below and to the side of the origin of the ventral mandibular muscle. (Fig. 1, F.)

There is a gland at the base of each stylet which in the adult is small. At the base of the maxilla it is usually L-shaped, extending laterally and dorsally, with the expanded bladeliike portion of the stylet forming a chitinous wall to part of the gland; at the base of the mandible it is more pyriform, lying above, and external to, the hollow chitinous rod. The cavities of these glands are continuous with the cavities of the stylets and sometimes show tissue inside the base of the cavities. These glands are apparently the homologues of the "retorten formigen Organen" of German authors.

In the third, fourth, and fifth nymphal stages the stylets are each connected with spiral coils of tissue lying in the prothorax, which are much more like the "retort-shaped organs" of aphids and other Homoptera. These coils and the connections with the stylets are hollow and are lined with a chitinous membrane except at the proximal end in the interior of the coil. This enlarged proximal end and some of the tissue surrounding the coil consist of a compact mass of quite distinct cells with enlarged nuclei. Toward the end of the fifth nymphal stage these structures degenerate entirely, and the glands at the base of the stylets in the adult are all that are left. The details of these structures are not included in this paper, as they apparently have no intimate relation to the digestive secretions of the insect.

BUCCAL GLANDS

Immediately above the place where the maxillae come together to form the suction and ejection canals lie certain interesting cells which may perhaps be best termed the "buccal gland cells." In this region, which probably represents all that is left of the mouth cavity in the Hemiptera, the dorsal plate, a continuation of the dorsal

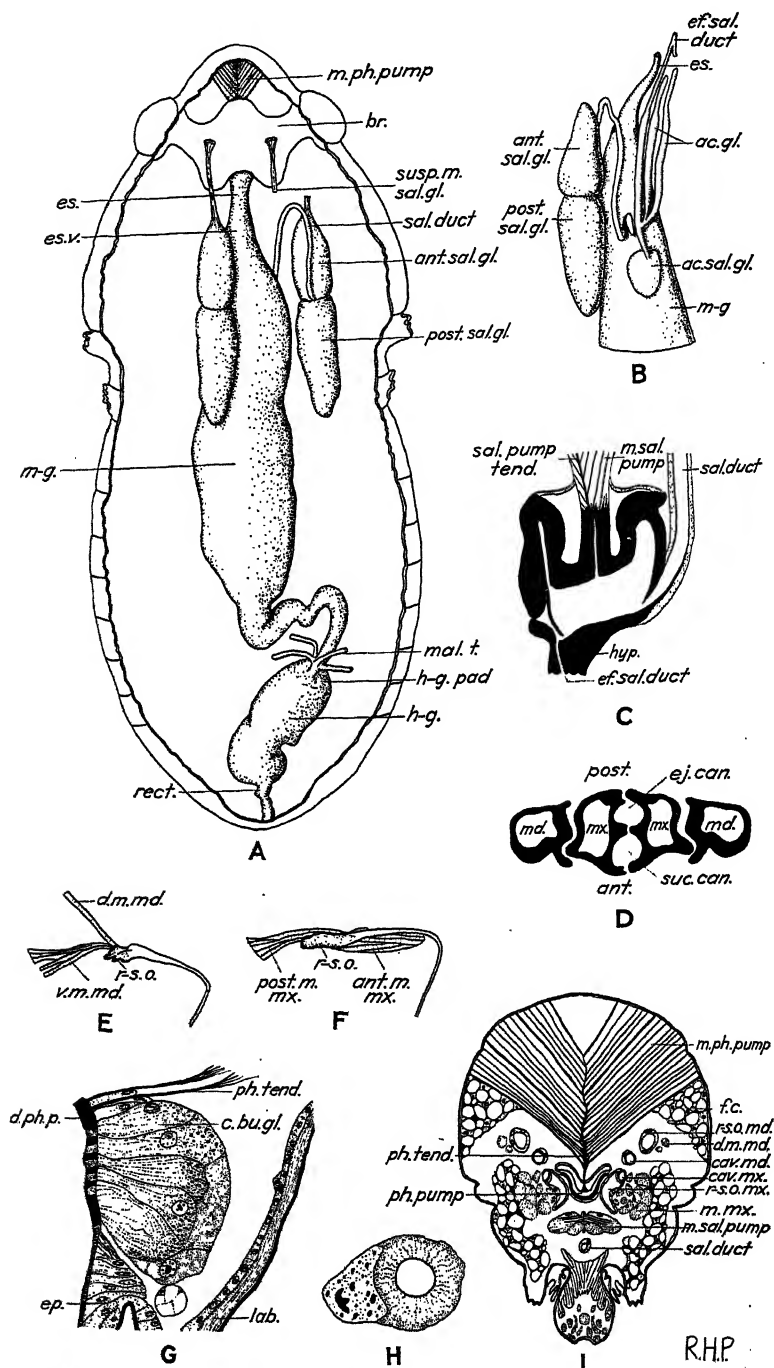


FIGURE 1.—The cotton flea hopper, *Psallus seriatius*. (For explanatory legend see opposite page)

pharyngeal plate, is perforated by two longitudinal rows of five pores each, above which lie the gland cells. A longitudinal section of these structures is shown in Figure 1, G. Near the pores the cytoplasm of these cells is sparse and fibrous; the distal ends of the cells, in which lie the nuclei, are filled with large granules that take haematoxylin stain quite deeply. Several cells containing cytoplasm of quite a different structure lie beyond the distal end of the gland cells proper.

The function of these glands is problematical, and probably they are too small for experimentation. Their secretion, which is of small quantity, mingles with the incoming food and may in some way take the place of the secretion of the salivary glands which, in other insects, open at about this point, since in this insect the salivary glands proper do not communicate directly with the alimentary canal. The buccal gland cells may be sensory in function, as for taste, but the writer has been unable to find a nervous connection of any size; moreover, these cells have the appearance of having the function of secretion.

ALIMENTARY CANAL

For the purpose of description the alimentary canal may be divided conveniently into the fore-gut consisting of the pharynx and the esophagus, the undifferentiated mid-gut separated from it by the esophageal valve, and the hind-gut. Like the mid-gut, the hind-gut shows little or no separation into distinct divisions.

The pharynx extends from the buccal glands back to a point almost beneath the center of the brain. In cross section it is horseshoe shaped and consists of a heavy chitinous ventral plate connected with a lighter dorsal plate by a region thinly chitinized. Surrounding this structure is a very thin layer of small epithelial cells; few or no muscular elements are to be seen, either as circular or longitudinal muscles. Piercing this epithelial layer and attached to the center of the dorsal plate is a comblike structure of erect chitinous tendons. This structure runs the entire length of the pharynx. From their point of attachment on the chitinous tendons, divergent muscles extend to the sides and are fastened to the vertex and front, appearing V-shaped

EXPLANATORY LEGEND FOR FIGURE 1

A.—General anatomy of the alimentary canal and salivary glands, drawn from dissection and sections $\times 107$; *ant. sal. gl.*, anterior salivary gland; *br.*, brain; *es. v.*, esophageal valve; *es.*, esophagus; *h-g.*, hind-gut *h-g. pad*, hind-gut pad; *m-g.*, mid-gut; *m. ph. pump*, muscles of salivary pump; *mal. l.*, Malpighian tubule *post. sal. gl.*, posterior salivary gland; *rect.*, rectum; *sal. duct*, salivary duct; *susp. m. sal. gl.*, suspensory muscle of salivary gland.

B.—Salivary gland, duct, and accessory gland, drawn from a dissection of a last-stage nymph; $\times 107$; *ac. gl.*, accessory gland; *ac. sal. gl.*, accessory salivary gland; *ant. sal. gl.*, anterior salivary gland; *ef. sal. duct*, efferent salivary duct; *es.*, esophagus; *m-g.*, mid-gut; *post. sal. gl.*, posterior salivary gland.

C.—Longitudinal section of the salivary pump, drawn from an unstained whole mount, $\times 893$; *ef. sal. duct*, efferent salivary duct; *hyp.*, hypopharynx; *m. sal. pump*, muscles of salivary pump; *sal. duct*, salivary duct; *sal. pump tend.*, salivary pump tendon.

D.—Cross section of stylets just after they emerge from the head, $\times 893$; *ant.*, anterior; *ej. can.*, ejection canal; *md.*, mandible; *mx.*, maxillae; *post.*, posterior; *suc. can.*, suction canal.

E.—Base of mandible with retort-shaped organ and muscular attachments, $\times 481$; *d. m. md.*, dorsal muscle of mandible; *r-s. o.*, retort-shaped organ; *v. m. md.*, ventral muscle of the mandible.

F.—Base of maxillae with retort-shaped organ and muscular attachments, $\times 481$; *ant. m. mx.*, anterior muscle of maxillae; *post. m. mx.*, posterior muscle of maxillae; *r-s. o.*, retort-shaped organ.

G.—Longitudinal section of buccal gland cells and pores, $\times 893$; *c. bu. gl.*, cells of buccal gland; *d. ph. p.*, dorsal pharyngeal plate; *ep.*, epipharynx; *lab.*, labrum; *ph. tend.*, pharyngeal tendon.

H.—Cross section of salivary duct, $\times 413$.

I.—Cross section of head just in front of the eyes, $\times 308$; *cav. md.*, cavity of mandible; *cav. mx.*, cavity of maxillae; *d. m. md.*, dorsal muscle of mandible; *f. c.*, fat cells; *m. mx.*, muscle of maxillae; *m. ph. pump*, muscles of pharyngeal pump; *m. sal. pump*, muscles of salivary pump; *ph. pump*, pharyngeal pump; *ph. tend.*, pharyngeal tendon; *r-s. o. md.*, retort-shaped organ of mandible; *r-s. o. mx.*, retort-shaped organ of maxillae; *sal. duct*, salivary duct.

in cross section. (Fig. 1, A and I.) The entire structure may be called the pharyngeal pump.

The esophagus extends from almost beneath the center of the brain, where it attaches to the pharynx proper, to a point near the middle of the prothorax, where it merges into the esophageal valve. In the esophagus the intima is formed as a direct continuation of the dorsal and ventral plates of the pharynx, but being very much thinner it is only barely visible under high-power magnification. The epithelial layer is extremely thin, but its cell walls may still be distinguished in good preparations. A few strands of longitudinal muscle extend the length of the esophagus, but these are very small. The circular muscles, basement membrane, and peritoneal membrane are either absent or too delicate to be visible. Four small muscles support the esophagus just back of the brain. Two tracheal trunks run alongside the esophagus through the circumesophageal connectives.

The esophageal valve is very simple and is well shown in Figure 2, B. At the junction of the mid-gut and fore-gut a ring of one or two cells secretes a peritrophic membrane which is short and disappears entirely only a very short distance below the entrance of the mid-gut. Except for these few cells there is an abrupt change from the structure of the fore-gut to that of the mid-gut.

The mid-gut extends from the esophageal valve into the posterior third of the abdomen with few curves and showing no definite morphological divisions exteriorly. The anterior part is usually much more distended than the posterior part and occupies the bulk of the body space in that region. There is a distinct coil to the right just above the pyloric valve in most specimens. The contents of the mid-gut have been studied and will be described later. Histologically, the mid-gut consists principally of large columnar epithelial cells which vary somewhat in size and shape, according to the distension of the stomach and the age of the cells. A loose lattice work of small strands of circular and longitudinal muscles covers the entire mid-gut, the former being on the inside. The longitudinal muscle strands are relatively few. There seems to be no peritoneal membrane. The enteric epithelium just below the esophageal valve consists of fairly regular cells with large nuclei, each cell with a distal striated hem. Farther down in the stomach wall the cells when they are young and small still possess this hem; as they get older they appear to lose it before discharging their secretory contents. These older cells give off droplike protrusions which extend into the lumen of the gut and later break off and discharge their contents. Relatively few small, young cells have been noticed, so that it is probable that secretion by any one cell is more or less continuous. In the living material the cells of the epithelium are often filled with what appear to be oil globules, which very often are seen to take up green coloring matter, presumably chlorophyll. This same color often appears in the cells of the fat bodies. In preserved material and sections these oil globules show as vacuoles of varying size. There seems to be no correlation between the size of these globules and the age of the cell or the condition of the individual. In many of the cells of some specimens there occur bodies of varying size which stain a deep black in iron haematoxylin. The possible nature of these will be discussed later. The remaining cytoplasm is fairly uniform. There seem to be no distinct differences or divisions in the kinds of

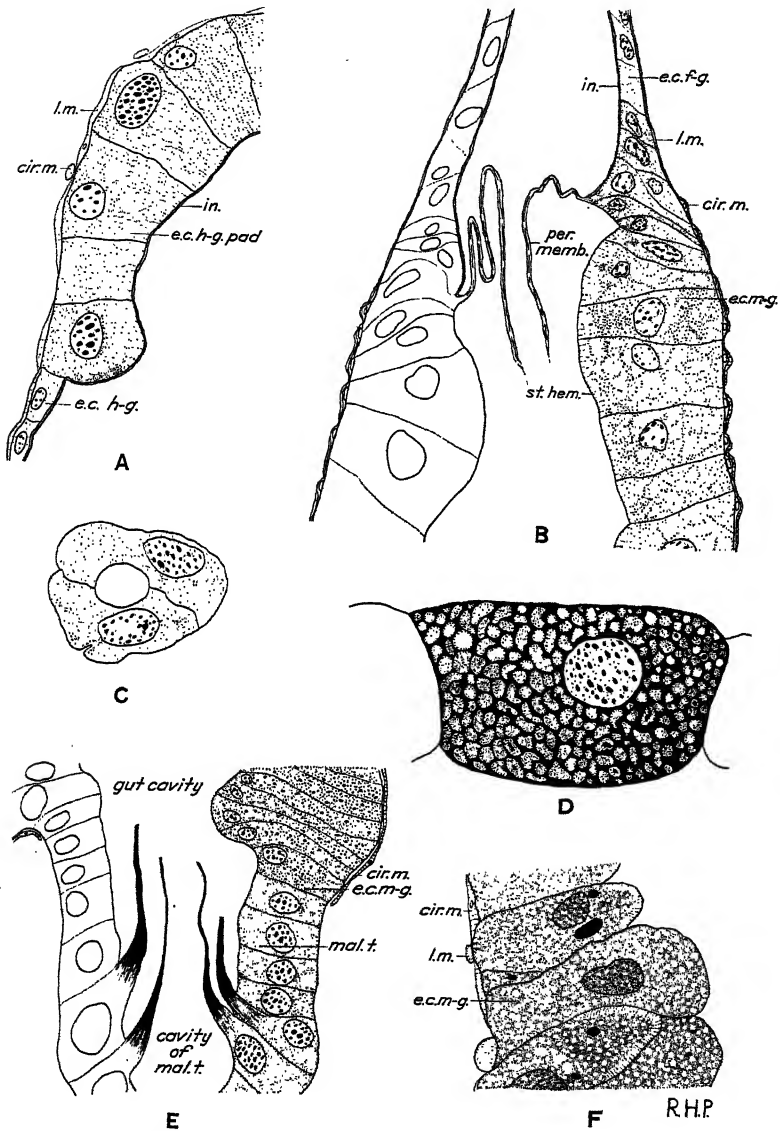


FIGURE 2.—Anatomical details of the cotton flea hopper, all about $\times 893$. A, Longitudinal section of cells of hind-gut pad and hind-gut wall; *cir. m.*, circular muscle; *e. c. h-g.*, epithelial cell of hind-gut; *e. c. h-g. pad*, epithelial cell of hind-gut pad; *in.*, intima; *l. m.*, longitudinal muscle. B, Longitudinal section of esophageal valve; *cir. m.*, circular muscle; *e. c. f-g.*, epithelial cell of fore-gut; *e. c. m-g.*, epithelial cell of mid-gut; *in.*, intima; *l. m.*, longitudinal muscle; *per. memb.*, peritrophic membrane; *st. hem.*, striated hem. C, Cross section of one of the anterior accessory salivary glands. D, Longitudinal section of a cell of the pyloric valve, showing entrance of a Malpighian tubule. E, Cross section of a part of the pyloric valve, showing entrance of a Malpighian tubule. F, Cross section of several cells of the mid-gut wall; *cir. m.*, circular muscle; *e. c. m-g.*, epithelial cell of mid-gut; *l. m.*, longitudinal muscle

cells found in any part of the mid-gut; they are uniform in appearance throughout unless changed by distension on account of the gut contents. Near the entrance of the Malpighian tubules the strands of muscle seem to be more numerous.

The pyloric valve is quite simple in this species. Just anterior to where the Malpighian tubules enter the alimentary tract a ring, two cells thick, of the enteric epithelium projects inward and upward. Between this place and their entrance the mid-gut is somewhat constricted. There seem to be no other structures of note in the valve. The structure of the entrance, however, shows some things of particular interest. The four Malpighian tubules join the gut at about equal distances around its circumference. Five or six cells back from the entrance of each tubule there are two circles of cells which give rise to chitinous projections, having much the appearance of the "flame cells." (Fig. 2, E.) Unlike the intima of the hind-gut, these projections stain a deep black with iron haematoxylin, as in the heavily chitinized stylets and pharyngeal plates. The projections have the appearance of being definite secretions of each of the cells and are long enough to extend out through the opening of the tubules and back into the lumen of the hind-gut.

The wall of the hind-gut, except for the hind-gut pad, shows very little difference in structure throughout its entire length and almost no external morphological differences. This portion of the alimentary canal extends from the opening of the Malpighian tubules to the anus, which opens on the ventral part of the last segment. The posterior part of the hind-gut is often somewhat constricted to form a kind of rectum, but there is no difference in the character of the wall. The hind-gut pad consists of a circular disk of tissue surrounding the pyloric valve just below the entrance of the Malpighian tubules and lies mostly on the dorsal side of the hind-gut. (Fig. 1, A.) The cells which compose it are relatively very large and contain large basal nuclei. (Fig. 2, A.) The cytoplasm is fibrous on the distal two-thirds of these cells and has a striated appearance under the microscope. The chitinous intima is somewhat thicker here than in the remainder of the hind-gut. The function of these cells is problematical. The remainder of the wall of the hind-gut consists of small cells similar to but more delicate than those of the fore-gut, with chitinous intima on the inside and a latticework of circular and longitudinal muscles on the outside. These muscles extend over the hind-gut pad. The circular muscles here are on the outside.

SALIVARY APPARATUS

The salivary glands are two roughly spindle-shaped structures lying on each side of, and dorsal to, the beginning of the mid-gut. A constriction, and internally a partition, divides each into an anterior and a posterior part. The size of the gland varies greatly in different specimens, apparently due to the increase or decrease of secretion and its subsequent use. When the glands are distended with the salivary secretions they extend even into the head above the brain and far back into the abdomen. The more usual conditions are represented in Figure 1, A and B. Fastened to the anterior end of each gland is a slender muscle which attaches it to the exoskeleton on each side above the brain. The cells of the salivary gland are very large, roughly hexagonal to square, with large nuclei.

(Fig. 2, D.) The cytoplasm takes a very dense stain with haematoxylin or other nuclear stain. The nuclei are lighter but contain darkly stained granules. In the dense cytoplasm are areas which are much lighter or even clear. When the gland is greatly distended the cells lengthen and widen, but are thinner, so that the nuclei protrude out into the lumen, though they are still covered with a thin layer of cytoplasm. This gives these cells a striking appearance; they differ greatly in general outline from the same cells when the gland is not distended. The secretion in the cavities shows up prominently in sections and is different in the two divisions. Throughout, the secretion is characterized by small droplets embedded in a material which takes up a stain. In the anterior part the secretion takes a nuclear stain; in the posterior part the secretion stains as does the cytoplasm. In addition the anterior part contains some larger vacuolated droplets which sometimes hang together in chains and have the general appearance of growing yeast cells. Although appearing to be without structure, they stain darker than the remaining secretion. (Fig. 3, A and B.) These vacuolated droplets are never seen in the posterior part of the salivary gland.

The salivary duct opens into both divisions of the salivary gland from the ventral side in the region of the partition. The exact course of the duct varies considerably in different specimens, but usually it is as shown in Figure 1, B. From its opening the duct passes forward dorsally to the alimentary tract to about the anterior end of the salivary gland, then posteriorly along the side of the mid-gut to a point about even with the middle of the posterior gland. Here it makes several coils upon itself, is joined by the accessory salivary glands, and passes forward toward the head again, around the circumesophageal commissure and beneath the optic nerve. Under the pharynx it is joined with the duct on the other side, which, in a very short distance, empties into the salivary pump. (Fig. 1, C.) In sections the salivary duct has the appearance of an intracellular tube and is lined with a chitinous membrane or has here at least a very thick cell wall. In longitudinal sections the nuclei are seen to alternate so that the duct forms a sinuous tube among them. In cross section the nuclei appear decidedly eccentric. (Fig. 1, H.) They are much fewer in number as the duct approaches the salivary pump. The cell walls are indefinite.

Three of what appear to be accessory salivary glands or reservoirs join the duct in the position shown in the figure (fig. 1, B), as previously described. Two of these tubular accessory glands pass forward and are often wound about the salivary duct; the third extends posteriorly for a distance varying in different insects and is frequently enlarged greatly to form a thin-walled disklike sack which is closely applied to the mid-gut. The other branches also vary somewhat in size and shape. In cross section these accessory glands usually have several cells and more than one nucleus visible in the same section. The cytoplasm of these cells (fig. 2, C) usually contains vacuoles and is much less dense than that of the cells of the salivary duct proper, which sometimes seem to have a pigmented area around the periphery. The center of these accessory glands rarely contains any secretion which stains definitely.

The salivary pump, which connects with the united salivary duct, lies immediately beneath the anterior end of the pharyngeal pump.

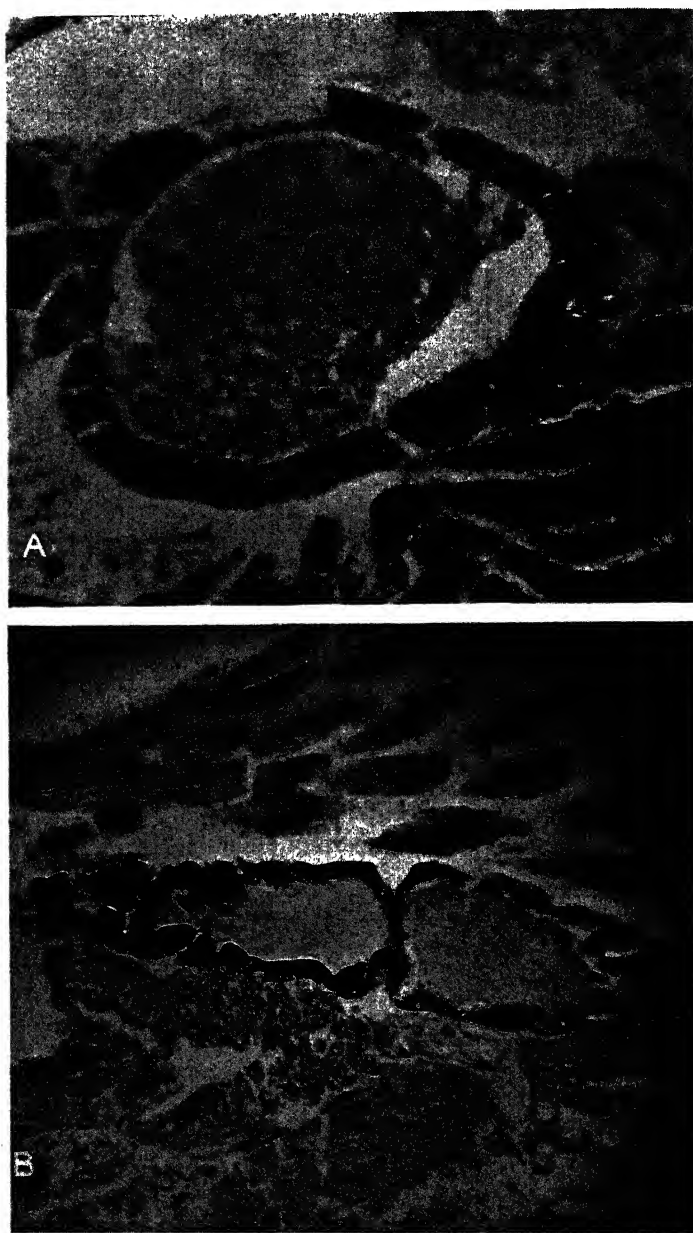


FIGURE 3.—A, a longitudinal section of part of the anterior part of the salivary gland of *Psallus*, showing the character of the secretion. Iron haematoxylin and orange G; B, a longitudinal section of the salivary gland of *Psallus*, showing the nature of the secretion of both parts. Ehrlich's haematoxylin and orange G.

It consists of a bell-shaped chitinous organ fitted against the proximal end of the hypopharynx. In the base or proximal end of the bell-shaped part is inserted the plunger of the pump, as shown in Figure 1, C. A chitinous tendon extends through the mass of muscles which work the plunger. These muscles at their middle are divided into two sections, each of which fastens to the head capsule just beneath the eyes. The salivary duct passes forward ventrally and empties into the front of the pump chamber. The dorsal lip of the opening of this duct probably acts as a valve when the plunger goes down, thus forcing the secretion out through a channel in the hypopharynx. A projection from the dorsal wall of the pump extends forward toward this efferent salivary duct and may act in some manner as a valve. The tip of the hypopharynx carrying the efferent salivary duct extends down into the ejection canal formed by the maxillae.

MECHANISM OF MOUTH PARTS USED IN PIERCING AND SUCKING

The mechanism of piercing and sucking has been thoroughly discussed by Grove (4) in a paper on *Psylla mali*, in which the various theories of previous writers as to how these things are accomplished have been thoroughly reviewed. Many of his statements on this homopteran may be applied here, except that the actions in this case are less involved. The insect by preference feeds on the more tender parts of the plants and, in the case of cotton, often chooses the red pigment glands. When the insect is ready to feed it elevates the front of the body and brings the beak perpendicular to the plant surface. The area is explored with the tip of the labium, and then the head is forced toward the surface of the plant. The labium bends caudad with the second joint from the base, forming at the point of the "V" an angle of about 30°. The stylets form the front of this V and extend through the other two sections of the labium, which, according to the writer's observations, never release them. The stylets are removed from the plant merely by raising the head, and another place for feeding is quickly chosen. As a rule the insects do not feed long in one place, but occasionally one is seen to remain in position several minutes. Often what are evidently punctures for exploration are made, and the stylets are driven in only a short distance and withdrawn. The entire beak is quite flexible, and a surprisingly large area can be investigated by means of the tip of the labium without further movement by the insect. In a study of sections of the plants on which the insects have fed, it has been noticed that the beak rarely penetrates farther than the first cells of the xylem.

The action of the salivary pump is relatively simple and in this insect is similar to that described for other Hemiptera. The large salivary pump muscles serve to pull back the plunger, thus creating a partial vacuum in the chamber and drawing in the salivary secretion. The elasticity of the chitinous membrane at the juncture of the plunger and the pump wall proper is probably sufficient to force the plunger back into place, close the inlet of the salivary duct, and force the salivary secretion out into the ejection canal through the hypopharynx. The secretion then passes out through the ejection

canal of the stylets. In life these insects may often be seen with a drop of semiopaque liquid, which is probably the salivary fluid, at the end of the beak.

The factors involved in drawing out the sap from the host plant along the suction canal, as they have been stated by Grove and others, may be briefly reviewed thus: (1) Capillary attraction up through the suction canal; (2) pressure of the sap or turgidity of the cells; (3) the pumping action of the pharynx accomplished by the raising of the dorsal pharyngeal plate, probably in a series of waves starting at the anterior end; (4) the peristalsis in the esophagus; and (5) the action of the esophageal valve. Although there seems to be no definite organic connection between the end of the pharynx and the suction canal, it seems probable from the size of the muscles and from the constancy of their appearance throughout the order that the pumping action of the pharynx is the principal factor involved. It is possible that the direction of the pumping force may be reversed, driving a secretion out of the gut into the plant in addition to the salivary fluid; but from the presence of a definite esophageal valve, although this is somewhat reduced in complexity, and from the lack of visible secreting cells of any size in the fore-gut, this would not appear to be a normal occurrence in this insect.

PARASITES

During the course of the study of the anatomy of the insect, parasites have been looked for which might possibly cause the deformation of the plant tissue. By making a smear of the entire mid-gut as soon as it is removed from the insect, it is possible to find a few coccus forms of bacteria. These are relatively scarce and are no more than might be expected, having been described in other insects in the same situation, and their presence here is probably not of importance in the general problem under discussion. Jenner's blood stain was used on these slides. The presence of some dark staining bodies has been described in the enteric epithelium of the mid-gut. (Fig. 2, F.) Three things with regard to these seem to point to their being metabolic products rather than parasites: (1) They vary greatly as to size and shape; (2) it is impossible to demonstrate anything but a homogeneous composition by means of stains; and (3) these bodies are found in the lumen of the mid-gut and hind-gut from the point where they begin to occur in the epithelium, about opposite the first abdominal segment, on through to the end of the gut in apparently undiminished size and number. It is suggested that these may be excretion products from these cells. The character of the secretions of the salivary gland in this insect has been described previously and presents some points of interest. The appearance of the smaller emulsoid bodies throughout the cavity of both the anterior and posterior division of this gland probably may be taken as the natural appearance of the staining reaction of the secretion in this insect. The appearance of the large globules in the anterior salivary gland is striking and peculiar in that it is limited to this section of the gland and was present in practically all the specimens examined, some 60 in all. (Fig. 3, A and B.)

STUDIES OF PRESERVED PLANT MATERIAL WITH RESPECT TO
THE EFFECTS OF THE BITE OF THE COTTON FLEA HOPPER

NATURE AND EXTENT OF COTTON FLEA HOPPER INJURY ON COTTON

So far as the external appearance goes, the effect of the *Psallus* bite upon cotton is threefold: (1) It causes a shedding of the young flower buds, or squares; (2) the number of internodes is increased while their length is decreased; and (3) conspicuous swellings appear at intervals over the whole plant but seem to occur either near, or exactly at, the point of the bite, the number of these bites and the persistence of the swellings giving rise to the apparent systemic condition. As a result of the first two, the entire habit of the plant is much changed, so that "hopper plants" may easily be recognized in any field. These plants are further characterized by having short branches excepting the lower two or three, which are often long; the plant itself is tall and spindling. The swellings appear on all parts of the stem, petiole, midrib, and the main veins of the leaf.

EXPERIMENTS ON HOPPERS AND ON LIVING COTTON PLANTS

During the summer of 1925 a number of experiments were undertaken at Port Lavaca, Tex., to determine whether the insect carried a plant disease or, if not, what were the main factors producing the result of the insect bite. Since material from these experiments has been used in the present study it is necessary to review the results of this work.

The experiments were principally attempts to duplicate the work of the hopper by artificial means. As the summer's work advanced it became more evident that the effect of the hopper's bite was quite local and apparently not transmitted through the plant itself from one part to another. So, while the first problem was kept in mind, the later work was directed toward the isolation of other possible factors involved in the production of abnormal plants as a result of hopper bite.

INOCULATIONS AND FIELD EXPERIMENTS

METHODS

The plants used in all experiments were chosen in the field. A certain amount of protection against hoppers was afforded by dusting the plants with sulphur, and in certain experiments by covering them with sacks. The work was somewhat handicapped in some phases by the lack of plants entirely free from all insect injury. The large number of plants used made it impossible to provide cages.

At first, three methods of inoculation were used: (1) Hypodermic injections in various parts of the plants; (2) cuts with a safety-razor blade dipped in various solutions; and (3) punctures with a dissecting needle likewise dipped in the solutions. Later, owing to the fact that the mechanical disturbance of the plant tissue obscured the effect of the solutions, these methods were largely replaced by the use of punctures made with pins known as "minuten Nadeln." The pins were set in a small piece of softwood, mounted on a handle for convenience in using, the tip of the handle being dipped in shellac to fix the pins in place. After each set of inoculations all instruments used were sterilized in boiling water and 90 per cent alcohol, and the minuten Nadeln apparatus was again dipped in shellac. The places of inoculation and other special methods used are described under

each experiment. In all cases where hoppers were used in the experiments, from 100 to 150 of the insects were crushed in about 3 c. c. of rain water.⁶ In most cases notes were taken at 24-hour intervals.

THE FIRST SERIES OF EXPERIMENTS

G. F. White and K. P. Ewing started the first series of experiments with 70 cotton plants before the writer's arrival. Later some of the inoculations and notes were made by the writer. In these experiments the razor blade, dissecting needle, and hypodermic needle were used on leaf veins, leaf petioles, square stems and branches, the main stem at various places, and the root crown. Suspensions of crushed hoppers, croton juice, crushed diseased cotton, and crushed mint were injected. None of the results were clear-cut, but some differences in the way the lesions healed were noticed. There was no evidence of any systemic disturbance of the plant or any more shedding of the squares than occurred among the checks, hoppers being present in small numbers in the field.

THE SECOND SERIES OF EXPERIMENTS

In the second series 60 cotton plants were inoculated in a series of 5 experiments of 12 plants each, solutions from croton, mint, and diseased cotton juice, and suspensions of crushed hoppers from croton and from cotton being used. Inoculations for each experiment were as follows:

Plant 1 (check): Water injected with a hypodermic needle.

Plants 2, 3, and 4: Solution injected with a hypodermic needle into the base of the terminal bud and on two upper square stems.

Plant 5 (check): Safety-razor blade dipped in water, and slits made in two upper leaf petioles and two upper square stems.

Plants 6, 7, and 8: Razor blade dipped in solution, and inoculations made in places similar to those of the check.

Plant 9 (check): Pin punctures (*minuten Nadeln*), two groups of three on each of two upper leaf petioles and on a second square stem, and one group of three on the upper square stem, the groups of three being a stated distance apart, the pins having been dipped in water.⁶

Plants 10, 11, 12: Pin punctures made as in check; pins dipped in solution.

None of the plants, except those treated with crushed hoppers from croton, showed any constant, definite distinction between the checks and the inoculated plants. In the case of the checks injured with a razor blade the cuts were clean and healed with a tan color. In the plants inoculated with hoppers from croton there was a growth of distinctive whitish cells from the sides and bottom of the upper leaf petiole of one, the lower leaf petiole of another (slight), and from all the cuts on a third plant. In the case of the pin punctures the checks showed dark holes at the points of puncture, sometimes with a slight swelling about them. In the plants inoculated with hoppers certain punctures became enlarged and burst open, showing a tan-colored

⁶ Distilled water free from metallic impurities was not available where the experiments were carried on. In view of this fact and in the absence of sterile plants in the field, it was necessary to depend on a difference in reaction between plants inoculated with rain water and those inoculated with other substances in rain water, rather than to expect the checks to be entirely free from injury. Rain water was therefore used throughout the experiments.

granular tissue beneath the skin. These slits extended sometimes for a millimeter or more on each side of the puncture. This condition appeared in many of the punctures on all three plants inoculated, especially on the leaf petiole and second square stem. The slits appeared between 48 and 72 hours after the time of inoculation. A single puncture on one of the plants inoculated with mint had a somewhat similar appearance. These developments are referred to in this paper as split lesions.

INJECTIONS OF SUSPENSIONS OF COTTON BUDS ON WHICH HOPPERS HAD BEEN CAGED

A hundred hoppers were caged on the extreme tip of each of three cotton plants for 24, 48, and 72 hours, respectively. At the end of that time the tips were removed, crushed in 1 c. c. of water and injected into 10 plants by pin punctures in the two upper leaf petioles and two upper square stems. A fourth plant was protected from hoppers for three days, examined and found free from hopper damage, and was crushed and injected as a control. The results are given in Table 1.

TABLE 1.—*Effect on cotton plants of injections of rain water containing soluble matter from crushed tips of cotton plants on which Psallus had been feeding, Port Lavaca, Tex., July, 1925*

[Ten plants were inoculated on each date and 21 inoculations were made per plant]

Date inoculated	Period tip was exposed to feeding of Psallus	Average height of plants July 10	Plants showing split lesions	Total split lesions	Average plant growth during—	
					First week after July 10	Second week after July 10
1925	Hours	Inches	Number	Number	Inches	Inches
July 7.....	24	18.05	8	40	4.2	4.75
July 8.....	48	19.95	10	45	3.2	4.85
July 9.....	72	17.15	10	62	3.4	4.55
Do.....	(*)	20.65	7	16	4.4	4.40

* Check tip; suspension was made from an uninfested tip.

In addition to the split lesions, similar to those caused by inoculation with crushed hoppers, there was a difference in the average rate of growth which was paralleled by results with hopper cages; i. e., a slowing up of the growth when hoppers were first introduced, followed by a slight increase.

EFFECTS ON COTTON PLANT OF CONTINUOUS EXPOSURE TO SUSPENSIONS OF CRUSHED HOPPERS AND HOPPERS CAGED ON LOCALIZED AREAS

Eight cotton plants in the field were protected from hoppers for over a week. After the experiments were started, however, some hoppers hatched out in two of the plants and caused hopper squares⁷ before they were found and killed. Cups of adhesive plaster were made about the stems of three of the plants; one of these was filled with water to serve as a check, two were filled with crushed hopper suspension, and slits were then cut in the stem below the level of the

⁷The term "hopper squares" is used to designate those squares which when very small darken and die in a way which is typical of the injury produced by *Psallus seriatus* and related insects on cotton.

liquid so that the plants might continually take up the solution from the cups. The liquid remained about the check and one treated plant for 12 hours and about the other plant for 48 hours. Two plants, one of them the check, on which live nymphs were found, showed hopper squares. The other plant showed no typical hopper squares. The pressure of the sacks over the plants may have caused some of the squares to die. Table 2 summarizes the results.

TABLE 2.—*Effect on cotton plants of exposing the stems to suspensions of crushed cotton flea hoppers, applied July, 1925, Port Lavaca, Tex.*

Plant No.	Period of exposure	Height at time of inoculation	Growth during—		Dead squares found during the experiment
			Week preceding inoculation	Week following inoculation	
	Hours	Inches	Inches	Inches	Number
104.....	12	20.5	6.5	3.0	0
105 (check).....	12	25.0	6.5	3.5	1
106.....	48	23.0	7.5	2.5	6

* The pressure of the sack over the plant may have caused some of the squares to die.

Five plants were used in a test to determine the effect of hoppers confined on a limited area of the plant. One of these was used as a check, and on the other four were fitted vials from which the bottoms had been removed. Into these vials 15 hoppers were introduced every other day and there confined so that they could feed only on the area beneath the vial. The vials were placed as close to the terminal bud as possible. Data concerning these plants are given in Table 3.

TABLE 3.—*Effect of confining cotton flea hoppers on limited areas of cotton plants, Port Lavaca, Tex., 1925*

Plant No.	Height of plant at beginning of experiment, July 10	Total growth of plant from July 10 to 17	Distance from center of vial to tip of plant on July 17	Growth of plant from July 17 to 24, measured from center of vial to tip	Total growth of plant from July 24 to August 1 ^a
	Inches	Inches	Inches	Inches	Inches
107 (check).....	20.5	4.0	5.00	1.50
108.....	19.5	3.5	1.3	1.65	1.05
109.....	13.0	6.5	1.0	1.00	0
110.....	11.5	3.5	1.9	1.80	1.50
111.....	19.0	5.0	2.6	2.10	.50

^a Limited growth is probably due to pressure of inclosing sacks.

^b Total growth of plant in this one case.

The plants showed absolutely no hopper squares and little, if any, difference in growth. The only evidences of the presence of the hoppers were the lesions beneath the vials.

CHEMICAL INTRODUCTIONS AND INOCULATIONS FOR THE STUDY OF LESIONS ON COTTON STEMS

Commercial diastase, pepsin, trypsin, and invert sugar sirup solutions were each introduced into five plants. On each plant the introductions with minuten Nadeln pins were in two groups of three on each of the two upper leaf petioles and second square stem and one

group of three on the upper square stem. The checks were five plants similarly treated with water. At the same time, partly to serve as checks and partly to serve in a study of lesions, five plants were inoculated from 17 to 21 times with crushed-hopper suspension, in groups of three each at intervals of about one-half inch. Table 4 gives a summary of the two experiments.

TABLE 4.—*Effect produced on cotton plants by injections of various chemicals and of crushed cotton-flea-hopper suspensions, Port Lavaca, Tex., 1925*

Material injected	Injections per plant	Plants showing split lesions	Total lesions	Average height of plants at beginning of experiment	Average growth per plant during first week after treatment
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Inches</i>	<i>Inches</i>
Not injected (check plants).....	21	2	2	12.5	4.7
Diastase.....	21	5	30	10.1	3.6
Pepsin.....	21	3	11	10.6	3.2
Trypsin.....	21	1	2	11.3	3.9
Invert sugar.....	21	0	0	13.5	3.1
Not injected (check plants).....	54-63	3	28	14.7	3.2
Crushed-hopper suspension.....	54-63	5	111	12.2	2.9

* Nearly all on one plant.

Diastase was the only reagent which showed any results comparable with those from the inoculation of crushed hoppers. With regard to the other plants, nearly all of the split lesions were in the third, fourth, and upper part of the fifth internodes below the bud; in other words, at about the place where the cells were ceasing to grow. This method of inoculation on the main stem seemed best.

INOCULATIONS OF CENTRIFUGED CROTON JUICE

A large number of tips of *Croton engelmanni* were crushed in water and centrifuged for five minutes. Only chloroplasts and particles of other plant tissue were thrown down. The yellowish liquid above was drained off, and five cotton plants were inoculated with it on the main stem at intervals of about 1 cm. Five plants were similarly inoculated with the thicker part of the solution. None of these plants showed a single lesion comparable with those caused by the crushed-hopper suspension.

INOCULATION OF CROTON PLANTS

A crushed-hopper suspension was used in inoculations on the main stem of four croton plants. The plants showed a slight darkening in the region of the inoculation, but neither swelling nor split lesions.

INOCULATIONS OF COTTON PLANTS WITH SUSPENSIONS OF CRUSHED HOPPERS

A large number of hoppers were crushed in water and that filtered through a Berkefeld filter No. W-20, 10%; the filtrate was then introduced into the main stems of five cotton plants. The residue was introduced, unfiltered, into the main stems of five other cotton plants. In order to determine the effect of letting the suspension stand for some time before inoculating with it, five plants were

inoculated in the main stem with freshly crushed-hopper suspension and three hours later five more plants were inoculated with the same suspension. The results of these two experiments are presented in Table 5.

TABLE 5.—Number of split lesions produced by inoculating cotton plants in various ways with suspensions of crushed flea hoppers, Port Lavaca, Tex., 1925

Material injected	Plants	Inoculations per plant	Split lesions
	Number	Number	Number
Filtrate from crushed hoppers *	5	54-63	7
Residue left after filtering the suspension of crushed hoppers *	5	54-63	45
Unfiltered suspension of crushed hoppers *	5	54-63	36
Suspension of crushed hoppers, 3 hours after preparation	5	54-63	3

* Freshly prepared.

It is noticeable that the number of split lesions was less on these plants which were large than on the younger plants used earlier in the season from this same patch with the same number of inoculations. (Table 4.)

INTERMITTENT INOCULATIONS

This experiment was started as soon as the late-planted young cotton began to put on squares. From August 7 to September 11, 10 plants were given twice a week from 3 to 9 inoculations with crushed hoppers on the two upper internodes of the main stem and on each branch. Five plants were similarly treated with water. These 15 plants and 5 others for comparison were measured twice weekly. During the first week in September the inoculations were discontinued on 1 check plant and on 4 of the other plants to test the recovery of the plants from the effect of the inoculations. Toward the end of the experiment from about 150 to 200 inoculations per plant were given twice a week. Table 6 shows the differences in plant heights. Four plants were eliminated during the experiment, owing to injury by the bollworm (*Heliothis obsoleta* Fab.).

TABLE 6.—Effect on growth of cotton plants of repeated inoculations with crushed flea-hopper suspensions during fruiting period, Port Lavaca, Tex., 1925

Treatment of plants	Plants treated	Average height of plants		Growth of plants from Aug. 28 to Sept. 11
		Aug. 7	Sept. 11	
	Number	Inches	Inches	Inches
Check (untreated)	5	6.9	31.0	9.4
Inoculated Aug. 7 to Sept. 11 with suspension of crushed hoppers	3	7.5	23.6	7.0
Inoculated Aug. 7 to Sept. 11 with water	3	7.5	24.5	6.6
Inoculated with suspension of crushed hoppers Aug. 7 to Sept. 1; recovery Sept. 1 to Sept. 11	4	7.6	25.3	8.0
Inoculated with water Aug. 7 to Sept. 4; recovery Sept. 4 to Sept. 11	1	7.5	25.5	8.0

In addition to differences in height, all the inoculated plants showed an average shortening of the internodes by about one-third of their length. All plants made a low scrubby growth with numerous branches, often two at each internode. In all respects save in the

appearance of lesions there was no difference between the crushed-hopper and the check inoculations. Hopper squares appeared on all plants in about equal numbers. There is little doubt that in all cases this injury of the squares was due to the presence on the plant of hoppers and other mirids.

The results of the experiments reported in Tables 1 to 6 may be summarized as follows: (1) Lesions could be studied best when inoculations were made up and down the main stem at short intervals; (2) there was no evidence in any experiment of persistent systemic disturbance transmitted very far from the point of inoculation; (3) positive results were two, the occurrence of swellings at the points of inoculations with a split showing peculiar abnormal tissue later on, and a shortening of certain of the internodes which were inoculated when young; (4) the latter occurred about as often in control plants as in inoculated ones and was least common on vegetative branches, like those coming out near the bottom of the plant; (5) swellings followed by split lesions occurred rarely on the control plants and were of some significance as they appeared similar to lesions caused by the hopper; (6) these split lesions occurred in numbers on cotton plants receiving inoculations with (a) crushed-hopper suspension, (b) crushed cotton tips on which the hoppers had been caged in large numbers, and (c) diastase solution; (7) a split lesion consisted of a decided swelling appearing around the puncture which about 48 hours thereafter split, sometimes as much as 4 mm., exposing a tan granular material beneath, which became darker with age; (8) so far as was visible externally, plants showed a complete recovery from any and all inoculations, except in the region of lesions, which did not develop further; (9) there was a decided variation in the response of different plants, both as regards number and size of lesions and shortening of the internodes at the sites of lesions; (10) lesions appeared to develop best on internodes 3 to 5, inclusive, on the main stem, although they appeared at almost any point inoculated, that is to say, they appeared usually at the place where the cells were just ceasing their growth; (11) croton plants did not show a response similar to cotton when inoculated with crushed hoppers; (12) hoppers might be caged on cotton plants and permitted to feed close to the buds without causing a shedding of squares; and (13) suspensions of crushed hoppers which had been filtered or allowed to stand for several hours gave few or no lesions when injected into the cotton plant.

LABORATORY EXPERIMENTS

VITAL DYE EXPERIMENTS

An effort was made through the use of vital dyes to determine whether the hopper transferred material from one plant to another. The method used was to place the petioles of young leaves in a solution of the dye until the veins were colored, place hoppers on these leaves, and after a short time transfer the hoppers to plants which had been deprived of chlorophyll by being kept in the dark. Aqueous solutions of the following stains were used: Eosin, Bismarck brown, Congo red, aniline green, gentian violet, acid fuschin, neutral carmine, methylene blue, neutral red, and several mixtures of two of the above. All the dyes went up into the plant tissues to some extent, gentian violet least, and methylene blue, eosin, and neutral red most. During

the course of the experiments the hoppers were examined by dissection, and methylene blue, eosin, and aniline green were observed in the gut and Malpighian tubules. The green stain also caused the gut wall to appear opaque. The eosin caused the death of the hoppers in from two to three hours. Upon examination after death the dye was found in the Malpighian tubules; before death it was seen in the gut. No stain was ever seen in the second plant on which the hoppers were subsequently caged, but these experiments led to those described below.

NATURE OF THE EFFECT OF HOPPER BITE ON THE PIGMENT SPOTS

In the work with hoppers and vital dyes it was noticed that places on leaves where the hoppers had fed retained, when the leaf was placed in Carnoy's solution,⁸ the red pigment spots or oil glands (9) normally characteristic of the cotton leaf, whereas the untouched spots were dissolved out. In the experiments with this reaction the bottoms were removed from vials and replaced with stiff paper in which openings of a definite size and shape had been cut. Hoppers in varying numbers were placed in the vials for varying lengths of time, while the opening which had been made in the paper bottom of each vial was placed successively over different parts of the plant.

The effect of the bite of hoppers on pigment spots did not spread outside the area exposed to them, no matter where the hoppers were placed. The material on which the hoppers had fed was fixed in Carnoy's and in Bouin's solution for microscopic study of the areas affected. In an attempt to determine the nature of the reaction, a number of other sucking insects feeding on cotton were caged on cotton leaves in a similar manner. The aphids and two species of Cicadellidae that normally fed on cotton gave no results similar to those from the hoppers, though relatively few of these insects were used. Three specimens of nymphs of a pentatomid and four different species of Miridae gave, however, a result that seemed identical with that produced by the hopper under similar conditions.

An attempt was made to duplicate the effect of the hopper bite artificially. Stems and leaves were punctured and treated with crushed-hopper suspension and solutions of diastase, trypsin, and pepsin, and allowed to remain overnight. When placed in Carnoy's solution none of the specimens showed a distinct preservation of the spots. Later pieces of leaves and petioles were punctured and placed bodily in suspensions of crushed hoppers and in diastase solution for varying lengths of time. The hopper suspension showed no effect, perhaps on account of its great dilution; but the material soaked four hours in the diastase solution showed the preservation of the pigment spots about the areas of punctures. The checks that had been soaked in water showed no effect. Lack of time prevented the trial of other chemicals.

SUMMARY OF LABORATORY EXPERIMENTS

So far as the transference of material, by regurgitation, from plant to plant was concerned, the experiments with vital dyes were negative, but further tests along this line seem warranted.

⁸ Owing to lack of absolute alcohol the Carnoy's fixing solution was made up with 95 per cent alcohol 1 part, glacial acetic acid 1 part, chloroform 1 part, corrosive sublimate to saturation. The material was washed in 70 per cent alcohol.

Methylene blue, eosin, and neutral red proved to be the most promising dyes for tests of this kind.

When hoppers were caged on a leaf it was found that the pigment spots or glands about their bites failed to dissolve out in Carnoy's solution, as did the other spots.

Similar results were given by pentatomid nymphs and four other species of mirids under similar conditions.

Leaves punctured and soaked in crushed-hopper suspension gave no results, probably owing to the great dilution.

Leaves punctured and soaked in diastase solution for a short time showed a preservation of spots about the punctures when preserved in Carnoy's solution.

IMMEDIATE EFFECT OF THE HOPPER BITE

The material which was used in the study of the immediate effect of the hopper bite consisted of sections of those leaves on which hoppers had been caged for varying lengths of time and had been restricted in their feeding to a small square area by means of an opening in a paper at the bottom of a vial, as previously described. Whether the insects had fed on the plant 3 hours or 24, there were no apparent differences in the stained preparations. In the cells the cytoplasm showed a tendency to plasmolyze throughout the area on which the insects had fed. The chloroplasts and nucleus seemed to have been broken up and scattered through the cell, giving it a decided homogeneity. Iron haematoxylin stained all of the cell contents very heavily, so that it was impossible to make out details. Other stains, such as Ehrlich's triacid mixture, Ehrlich's acid haematoxylin, and safranin, gave somewhat better results but failed to resolve the cell contents into constituent parts. In this respect all the cells of the leaf in the square area on which the insects had fed reacted similarly. Cell walls in places both inside the leaf and on the outer epidermis had been ruptured in various places. These breaks had the appearance of being made mechanically rather than by any dissolving action. There seemed to be no tendency in this tissue for the effect of the bite to spread beyond the confines of the square in which the insects had fed, the cells on its edge being entirely normal.

In connection with this part of the study, sections were made of leaves which had been punctured and soaked in diastase solution for four hours. These sections in places around the punctures showed a clumping of the cytoplasm and dissolution of the chloroplasts similar to that of the material described above. The cytoplasm of these cells stained a deep black in iron haematoxylin. In one place noted about 15 palisade cells were affected on each side of the point of puncture. This may perhaps be taken as the average of spread in this length of time.

Relatively little could be ascertained concerning the effect of the insect's feeding on the pigment spots or glands. In the area not touched by the insects these appeared as circular openings surrounded by cells of an epithelial nature. In the glands on which the insects had fed or which had been touched by the diastase solution, there was a black staining material in the center of the gland and throughout the surrounding cells. The chemistry and anatomy of these glands have recently been studied by Stanford (10). It is interesting to note that the tissue on which the insects had fed was somewhat similar

in reaction and appearance to that found surrounding the beak of certain aphids when this organ was inserted into a plant. This has been described and figured by Wells (12) and Biedermann (1, pp. 808-810), and it has been shown that this sheath about the proboscis consists of calcium pectate and tannic substances.

A STUDY OF THE SWELLINGS AND RELATED INJURY CAUSED BY THE BITE

Externally the swellings caused by the bite itself showed little variation except as regards size, extent, and amount of splitting. On the plant they occurred on the stems, petiole, midrib, and smaller leaf veins. The swellings themselves were principally the result of an increase in size of the individual cells, and to a very slight extent of an increase in the number of cells. The swelling might take place in all directions but was always more obvious in cross than in longitudinal sections, hence laterally rather than with the axis of the stem. In the center of the swelling, apparently at the place where the insect bites, there was a distinct destruction of some of the cell elements, while others enlarged to take their place. Along the margin of an area of destruction certain cells seemed to have collapsed. Sometimes as a result of this destruction of cells, and at other times apparently without visible cause, there was a displacement of the position of normal cells. This was especially true of the elements of the vascular bundle, the position of the xylem often being changed as shown in Figure 4, A, and the constituent parts broken up as shown in Figure 4, B. The chief change in cell structure took place in the cortex and in the cells recently formed in the cambium, but the phloem was also involved, and less commonly the xylem. The individual cells showed a great diversity in form, usually being somewhat irregular and thus different in shape from those of normal condition.

In certain cases the structures of the cells themselves were changed. The epithelial cells were often found to have grown larger and varied in shape and size, appearing like those of the cortex or even more irregular. Very often certain elements had enlarged and taken the place of destroyed tissue, as is shown in the cross section of the midrib. (Fig. 5, A.)

The most marked change, however, was in the content of the cell wall about an area where the cells had been destroyed, or in a lesion which had split so that the cells were exposed to the air. Here the cellulose had disappeared from the cell walls and pectic material alone apparently survived. This was evident when such a section was examined under polarized light, and it was also shown by stains. This change in structure seemingly gave rise to the characteristic appearance of the split lesions seen in field work. In these structures the swelling split open, and the enlarged cells appeared as a tan granular substance in the wound. In section these split lesions were seen to be lined with dead cells, and the open part often extended back under the epidermis for a short distance in all directions, sometimes leaving pockets of destroyed cells overlaid by living ones. Some swellings which showed no external split had below the epidermis an extensive pocket of dead cells and often relatively large air spaces.

Frequently the entire contents of injured cells were lacking. But when still present there was always a most striking change of appearance in these parts. When stained with iron haematoxylin and

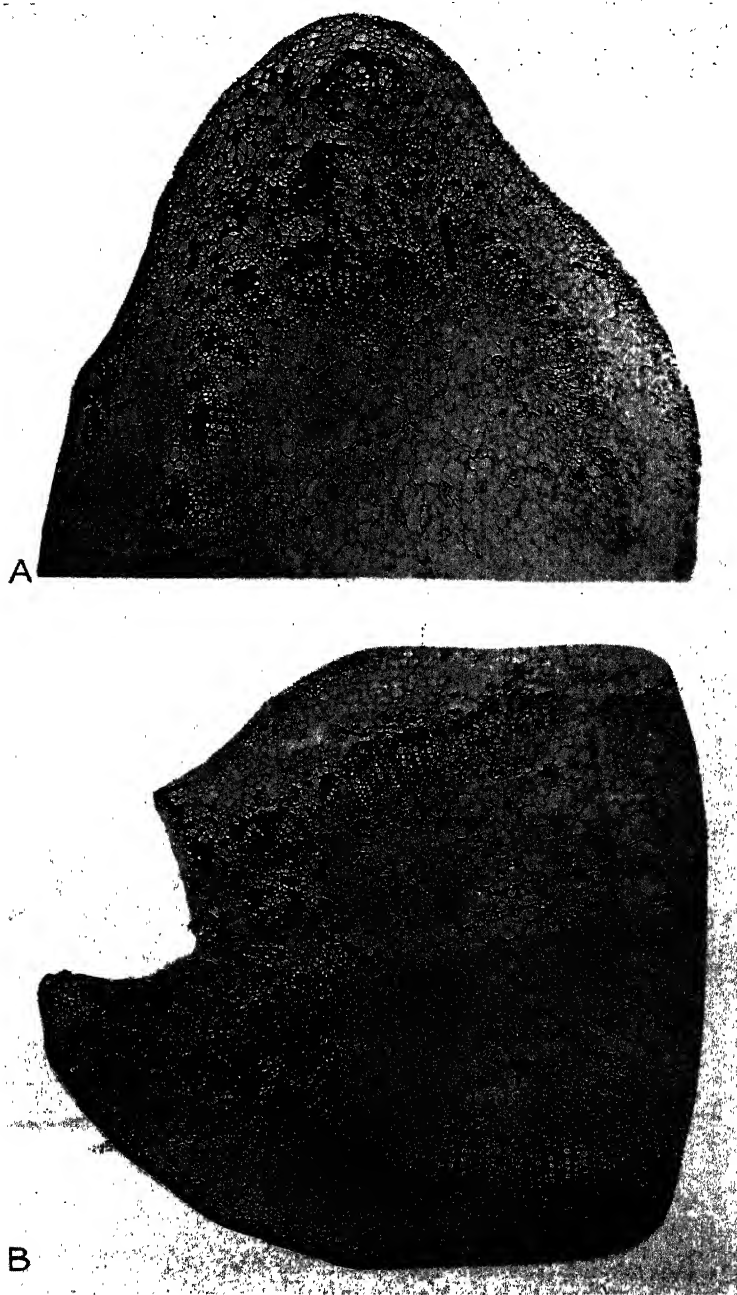


FIGURE 4.—Injuries to cotton by the cotton flea hopper, *Psallus seriatus*: A, Swelling and misplaced vascular bundle on stem caused by the insect; B, cross section of split lesion on stem caused by hopper bite. Material from hopper cages, Port Lavaca, Tex. All the photomicrographs of plant material used in the illustrations of this article were of sections stained with iron haematoxylin and orange G

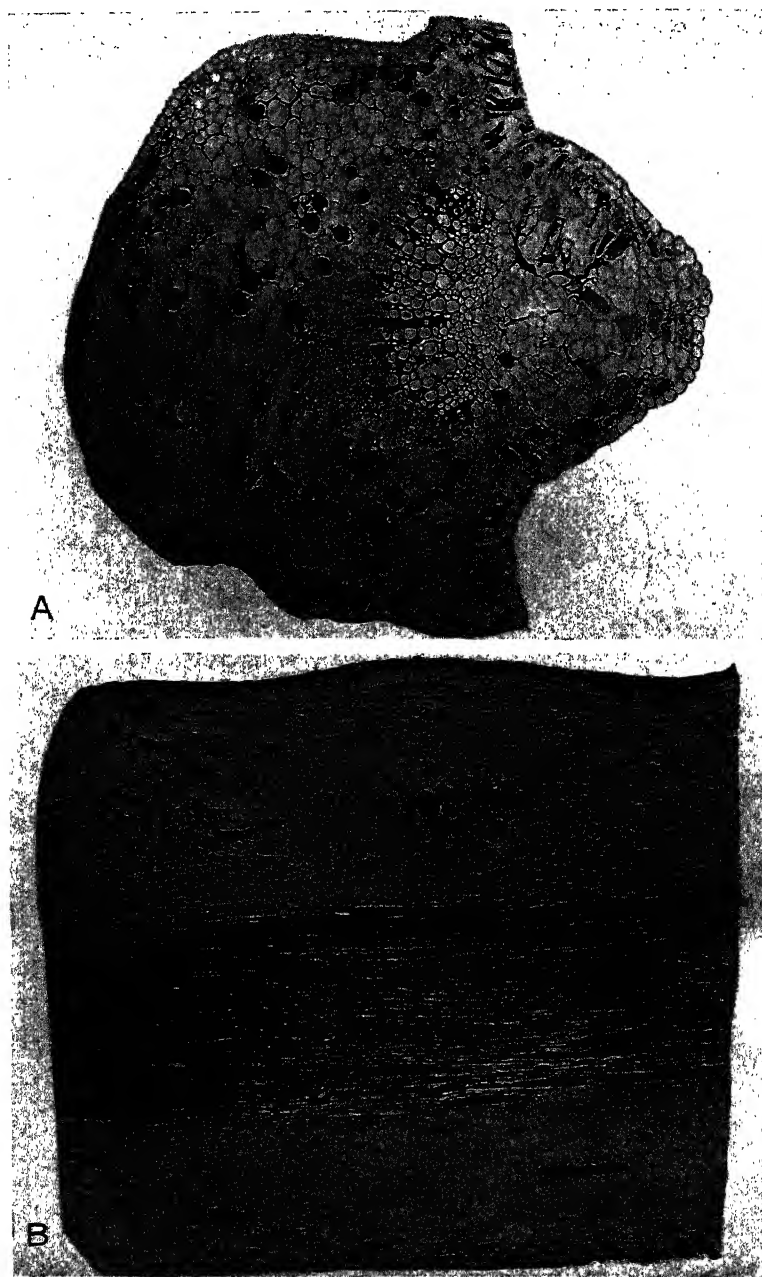


FIGURE 5.—Injuries to cotton by the cotton flea hopper, *Psaltus seriatus*: A, Cross section of swelling on midrib of cotton leaf; B, longitudinal section of stem preserved four days after the hoppers had been caged on the plant, showing a series of cells containing granular material which came from near the beginning of the region of the swelling. Material from the hopper cages at Port Lavaca, Tex.

orange G, the cells could be separated into four types, which seemed to merge into each other. Certain cells were filled with a homogeneous material which stained a deep black and showed no trace of nucleus or chloroplasts. Other cells were filled with a homogeneous yellow-staining material but with the nucleus still in the cell and the chloroplasts intact around the periphery. Other cells were filled with yellow particles of the same size in any one cell, but of different sizes in different cells. In certain of this group of cells a nucleus, or the chloroplasts, or both, were still visible. The last group of cells to be noted was that in which the cells contained oval or round particles, often with darker rim, and had a glistening appearance much like spores of fungi. These varied in different cells from the size of the largest yellow particles to about one-half larger. At times they were arranged in a loosely branching series throughout the cells. At other times they were arranged around the periphery of the cells. An average size for the largest was about 0.005 mm. (Fig. 5, A.)

The exact extent of the effect of the bite up and down the stem has not been ascertained. Experiments during the summer of 1925, however, point to the probability that it is fairly local, at least not extending more than two or three internodes from the point of bite. A very interesting fact concerning the material found in the affected cells about the lesions was that the cells involved tend to occur in vertical series, each series often separated from the next by several normal cells. There was a tendency at times to spread subcortically and also around the epidermis.

Material which had been inoculated with crushed-hopper suspension and later preserved and examined microscopically showed throughout effects which were similar in all cases to those due to the punctures by the hopper. The type of swelling and the change in cell structure and in cell contents were closely parallel in both cases. Figures 5, B, and 6, A, show this comparison very well. By microscopic examination it was also found that the method of inoculation with "minuten Nadeln" was quite satisfactory, the pin pricks extending only through the cortex, and hence very little farther than the proboscis of the insect entered.

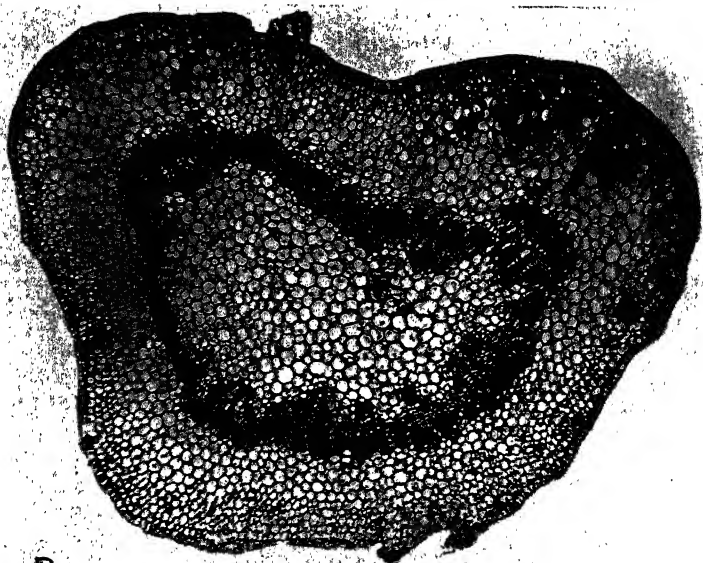
SHEDDING AND INSECT INJURY

A study of the literature on abscission in cotton shows that two theories have been advanced in regard to the probable cause of the shedding of the squares and bolls. Lloyd (?) has brought together considerable material to support his contention that abscission is a result of competition between bolls for water. Mason (8) has given support to the theory that the shedding is determined by the rate at which food is elaborated by the plant and is used by the developing fruit. The exact details of the method of abscission have not been completely worked out. It has been shown, however, that abscission is preceded by a growth of cells in the abscission zone, followed by a digestion of the middle lamella and adjacent layers of cellulose wall. Pockets of resin or gum inclosing loose cells are frequently present.

It will be seen from the preceding description of insect injury that the probable nature of abscission due to the insect may support either theory, or both, or neither. It is possible that a change in the cell wall or in cell contents will start the abscission reaction. It has



A



B

FIGURE 6.—Injury to cotton plant by the cotton flea hopper: A, Longitudinal section of stem through place of inoculation with crushed-hopper suspension, preserved 48 hours after puncture; B, cross section of stem in which the feeding place of the insect appears black, preserved after the insects had fed on the plant for 24 hours

been noted that a destruction of cells sometimes takes place at the site of the insect bite without being visible externally. This actual destruction of cells may in itself be the cause of the shedding of the squares or it may operate to prevent the absorption of water by the developing bud. The change in cell contents may have a similar result.

Preserved material has been examined to see whether it shows any correlation between the length of time during which the insects have been allowed to feed and the changes in the plant tissue. Four days after hoppers have been caged on a plant a cross section of the bud shows all stages of cell secretion, and in a longitudinal section all variations may often be seen in a single row of cells, so that it is impossible to point out any one cell as being the one in which the change originated. On one occasion insects were caged on a bud for 24 hours and then removed and the bud preserved in fixing fluid. On examination sections of this bud showed the cell contents in the condition previously described as containing yellow particles, and in other places the cells contained the black particles. (Fig. 7, A.) Where each locality represented a separate place of bite there was no admixture of the two types of cell contents. Certain cells were entirely emptied of their contents, and in others there was a clumping of the material of the cell, which stained very darkly, paralleling the condition described above. In the stem of this same bud a developing egg was found which was probably that of *Psallus*. The only evidence of injury in the region about the egg was a collapse of some of the cells. A typical section is shown in Figure 7, B.

CELL INCLUSIONS

In a study of this kind the possibility of the presence and transmission of organisms of a parasitic nature must be taken into consideration. The change in the character of the cell contents is the only fact derived from microscopic study which would point to the possibility of such organisms being present. It seems impossible that these peculiar structures are artifacts due to fixation, since a large number of fixing fluids have been used under various conditions. However, it may be that they are the normal regeneration responses of the plant to a wound of any kind. Other than this, there are four possible explanations for the change in appearance in the cell contents: (1) The structures seen may be gums or resins due to excess food, the products of insect secretion injected into the plant; (2) they may consist of a deposit of material by the plant in direct response to toxins or enzymes injected by the insects; (3) they may represent a developing parasitic organism showing stages of growth, and may be any of the following—yeast, slime mold, fungi, amoebae, or bacteria. In general appearance the diseased portion resembles a similar portion of the club root of cabbage. Certain stages show a likeness to developing yeast, and in this connection it is interesting to remember the similar appearance of the bodies in the salivary glands in the insect. (4) A part of the appearance may be due to a cell product induced by the developing parasite.



FIGURE 7.—A, Three feeding places of the cotton flea hopper on a bud of cotton, the plant having been preserved after the insects had fed on the bud for 24 hours; B, developing egg of *Psallus seriatus* in cotton bud, plant tissue otherwise normal

A STUDY OF FRESH MATERIAL OF THE COTTON FLEA HOPPER
AND OF INFESTED COTTON

METHOD OF DISSECTION

During July, 1926, an attempt was made to get further information with regard to the exact method by which the cotton flea hopper injures the plant. It was found possible to take out entire the salivary glands of *Psallus*. The insects were placed on their sides in a watch glass and were completely covered with a thin layer of paraffin which covered the bottom of the glass and which had been thoroughly impregnated with powdered charcoal. It was easily possible then to cut away the chitinous body wall from both dorsum and side, exposing the alimentary canal and other organs.

The anterior part of the salivary gland is nearly transparent, with more opaque places which represent the nuclei of the cells. The posterior part of the salivary gland is usually white and opaque, and on dissection this appearance proves to be due to the secretion in its cavity, the walls of the gland being semitransparent, as in the case of the other half of the gland. Specimens have been found in which the entire gland was transparent. On the whole it is difficult to see these glands except against the black background of the dissecting dish.

TESTS FOR THE PRESENCE OF AMYLASE

In making tests for the presence of enzymes, the method used by Swingle (11) was followed, except that the starch solution was diluted to about 10 per cent. In the case of each test for the presence of amylase, a few drops of the mixture of starch colored with I-KI solution was placed in the center of each of two concave slides. The two glands from a single specimen of *Psallus* were removed, one placed on each slide and thoroughly torn to pieces. One slide was then heated until the drop of solution boiled, destroying the enzymes present. This slide was used as a control. Both were incubated at 35° C. for 48 hours. In a second test the glands were treated in a similar manner but without the addition of I-KI, and after incubation the resulting solution was tested for the presence of reducing sugars by means of Fluckiger's reagent. The tests were repeated twice, but in no cases was there any indication of the digestion of the starch. The tests may be taken as an indication of the absence of amylase, but the results should be checked in future by other methods, as the glands are extremely small. Amylase might, moreover, be secreted by one of the accessory salivary glands.

In another series of tests the insects were allowed to feed on a restricted area of the stem by means of the vials described in a preceding paragraph. Free-hand sections were cut through this part of the stem and tested for the location of the reducing sugars, glucose and fructose, by means of Fluckiger's reagent (11, p. 215). There was no distinguishable difference between the part of the cortex on which the insects had fed and the part which had been protected from them. Tests were made on material on which the insects had fed for 24, 48, and 72 hours, respectively. These tests seemed to show that injury to the plant was not due to a starch-digesting enzyme proceeding from the salivary glands.

EXAMINATION OF MATERIAL IN CELLS OF INFESTED PLANTS

In an effort to determine the nature of the material found in the cells of infested plants, free-hand sections were made of a number of lesions found in the field and also in cages where only one species of insect had been introduced. Affected plants taken from fields at Scott, Miss.; Tallulah, La.; and Port Lavaca, Tex.; and from cages from the two latter places, have been examined. In all cases an abundance of material similar to that noticed in the preserved plants has been found. Considerable difficulty has been experienced on account of the low visibility of the material in the cells of the fresh plants. The cells which are affected seem under the microscope to have a homogeneous grayish, opaque appearance and little or no detail can be made out. If I-KI solution, such as is used in testing for starch, is placed on the section, certain of these cells stain a homogeneous yellowish brown and others are seen to be packed full of nonstaining, round or oblong, glistening bodies of various sizes in different cells. Swellings on the midribs of leaves seem to afford the best material for a study of these objects in the cells. Their appearance does not differ, except as regards color, from that of preserved material previously described. Sections of stems which had swellings were treated with Fluckiger's reagent, and these showed a larger quantity of glucose and fructose in the area of the swelling than elsewhere. This was often concentrated in the cells which contained the granular material. Whether this is due to a peculiarity of this material or to the malformation of conducting tissue can not be determined at this time. The granular material in the cells has also been found in swellings from plants on which the tarnished plant bug (*Lygus pratensis* L.) had been caged.

EFFECTS OF THE HOPPER BITE ON PLANT CELLS

In order to study the immediate effects of the hopper bite, several vials containing the insects were fastened against the stems of protected plants and the insects allowed to feed through openings in the paper which replaced the bottom of the vial. A vial was removed on each successive day, and sections were cut out of the stem beneath it. These sections were examined both in water and in I-KI solution. The area on which the insects had fed contained many cells which were empty, their contents being replaced by air. In one case whole masses of cells were absent, but this was possibly due to injury during cutting. In all this material small oval or round bodies were found in the plant cells. These bodies were glistening and moved about freely in the cell or often in the water near a ruptured plant cell. They varied in number from one to a dozen or more per cell and seemed to increase in number with the increasing number of days on which the hopper had fed on the plant. It has not been possible to ascertain whether these bodies have a definite relation to the granular material which later appears in the cells of the plant. Sections have been examined of material on which the insects had fed from one to five days. No information regarding the origin of the granular material in the cells, however, has been gained by these examinations.

SUMMARY OF STUDIES WITH FRESH MATERIAL

Tests failed to reveal the presence of a starch-digesting enzyme in the salivary gland of *Psallus*, but the tests were not conclusive, and the accessory salivary glands were not tested.

Infested cotton plants from Mississippi, Louisiana, and Texas have been examined by means of free-hand sections.

In all cases material was found in the cells which resembled that found in the stained sections of plants from Port Lavaca, Tex.

Oval or round glistening bodies were found moving about in the cells of plants on which the *Psallus* had recently fed.

The observations suggest the possible presence of an organism which is transmitted by the cotton hopper but which does not penetrate far from the point of introduction.

This assumed organism may be transmitted by *Lygus pratensis* also.

The appearance of the cell inclusions studied suggests that of a myxomycete or slime mold, such as the one causing club root of cabbage.

GENERAL SUMMARY AND CONCLUSIONS

The primary purpose of this study is in the nature of foundation work for future studies. The anatomy and histology of the alimentary canal of the cotton flea hopper, *Psallus seriatus*, are given. The structure of the salivary glands and their accessory glands and ducts has been worked out, together with the structure of the mouth parts. The presence of bodies which may be parasites in the anterior part of the salivary gland is discussed.

The effect of the feeding of the cotton flea hopper on the plant cells has been described, and a study of the tissues of infested cotton has shown, in addition to the malformations of the cells, the presence of cell inclusions near the site of the puncture. In certain preparations these cell inclusions have the appearance of an invading or developing parasite and have been found in both fresh and preserved plant material.

The field experiments have shown that the inoculum or material injected by the cotton hopper does not spread far from the point of injury. The appearance of a systemic disturbance, sometimes observed in the infested fields, therefore, seems to be due to the multiplicity of bites, and the shedding of the hopper squares seems to be due to a bite near by.

The secondary purpose of the paper is to call attention to possible plant-disease transmission by *Psallus* and to present the evidence that has been gathered. Further work along this line must be done by the collaboration of a plant pathologist and an entomologist, and should include in addition a study of the feeding of other Miridae on cotton.

LITERATURE CITED

- (1) BIEDERMANN, W.
1910. DIE ERNÄHRUNG DER INSECTEN (HEXAPODA). NEUNTER TEIL.
In Winterstein, H., Handbuch der Vergleichenden Physiologie.
Bd. 2, Erste Hälfte: [726]–902, illus. Jena.
- (2) CHAMBERLAIN, C. J.
1924. METHODS IN PLANT HISTOLOGY. 4th rev. ed., 349 p., illus. Chicago.
- (3) DUFOUR, L.
1833. RECHERCHES ANATOMIQUES ET PHYSIOLOGIQUES SUR LES HÉMIPTÈRES, ACCOMPAGNÉES DE CONSIDÉRATIONS RELATIVES A L'HISTOIRE NATURELLE ET A LA CLASSIFICATION DE CES INSECTES.
Paris Acad. Roy Sci. Mem. . . des Savans Etrangers. T. 4, p. [131]–461, illus. Paris.
- (4) GROVE, A. J.
1919. THE ANATOMY OF THE HEAD AND MOUTH PARTS OF PSYLLA MALI, THE APPLE SUCKER, WITH SOME REMARKS ON THE FUNCTION OF THE LABIUM. Parasitology 11: 456–488, illus.
- (5) HUNTER, W. D.
1924. THE SO-CALLED COTTON FLEA. Jour. Econ. Ent. 17: 604.
- (6) IMMS, A. D.
1925. A GENERAL TEXTBOOK OF ENTOMOLOGY, INCLUDING THE ANATOMY, PHYSIOLOGY, DEVELOPMENT, AND CLASSIFICATION OF INSECTS.
698 p., illus. London.
- (7) LLOYD, F. E.
1920. ENVIRONMENTAL CHANGES AND THEIR EFFECT UPON BOLL-SHEDDING IN COTTON (GOSSYPIUM HERBACEUM). Ann. New York Acad. Sci. 29: 1–131, illus.
- (8) MASON, T. G.
1922. GROWTH AND ABSCISSION IN SEA ISLAND COTTON. Ann. Bot. [London] 36: 457–484, illus.
- (9) SNODGRASS, R. E.
1927. THE HEAD AND MOUTH PARTS OF THE CICADA. Ent. Soc. Wash. Proc. 29: 1–16, illus.
- (10) STANFORD, E. E., and VIEHOEVER, A.
1918. CHEMISTRY AND HISTOLOGY OF THE GLANDS OF THE COTTON PLANT, WITH NOTES ON THE OCCURRENCE OF SIMILAR GLANDS IN RELATED PLANTS. Jour. Agr. Research 13: 419–436, illus.
- (11) SWINGLE, H. S.
1925. DIGESTIVE ENZYMES OF AN INSECT. Ohio Jour. Sci. 25: 209–218.
- (12) WELLS, B. W.
1920. EARLY STAGES IN THE DEVELOPMENT OF CERTAIN PACHYPSYLLA GALLS ON CELTIS. Amer. Jour. Bot. 7: 275–285, illus.

TWO NEW SPECIES OF NODULAR WORMS (OESOPHAGOSTOMUM) PARASITIC IN THE INTESTINE OF DOMESTIC SWINE¹

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INTRODUCTION

Before 1925 nodular worms occurring in the large intestine of domestic swine were considered a single species, *Oesophagostomum dentatum*. In 1925 Goodey² described a new species of this genus, collected in New Guinea from the intestine of a pig, presumably *Sus scrofa domestica*, and named it *O. longicaudum*. Shortly after the appearance of Goodey's paper, the senior author³ reported the presence of *O. longicaudum* in domestic swine in the United States, the Philippine Islands, French Indo-China, and the Fiji Islands based on a study of specimens in the helminthological collections of the Bureau of Animal Industry.

A recent study of a small collection of *Oesophagostomum* from domestic swine obtained by E. W. Nighbert, of the Bureau of Animal Industry in Moultrie, Ga., at the request of the senior author, revealed the presence of *O. dentatum* and *O. longicaudum* and of two additional species of this genus heretofore undescribed. One of these species (for which the name *O. brevicarudum* is proposed) was present in fairly large numbers, whereas the other (for which the name *O. georgianum* is proposed) was represented by 6 specimens, of which 4 were males and 2 females, one of the latter being immature.

DESCRIPTION OF SPECIES

Oesophagostomum brevicarudum, new species.

The mouth opening is in the center of the mouth collar, which is surrounded by an external leaf crown composed of from 14 to 16 elements and an internal leaf crown composed of from 28 to 32 elements. (Fig. 1.) In 8 specimens in which the head was studied in frontal view the following numbers of elements in the leaf crowns were noted: 5 males showed 14, 15, 16, 16, and 16 elements in the external leaf crown and 28, 30, 32, 32, and 32 elements in the internal leaf crown, respectively; 2 females showed 14 elements in the external leaf crown and 28 elements in the internal leaf crown, and 1 female showed 16 elements in the external leaf crown and 32 elements in the internal leaf crown. The elements of the external and internal leaf crowns are triangular in shape; those of the internal leaf crown are located at the base of those of the external leaf crown. The elements of the latter project a little beyond the mouth opening. (Fig. 2.) The head bears four submedian papillae and two lateral papillae or amphids. The buccal capsule is small, its walls, as seen in optical section (fig. 2), having a morphology characteristic of this species and consisting of a more or less triangular apical portion and a more or less spherical basal portion; the two portions are separated by an indentation that is more pronounced on the outer than on the inner margin. The cuticle between the cephalic groove in front and the cervical

¹ Received for publication Nov. 13, 1929, issued March, 1930.

² GOODEY, T. THE ANATOMY OF OESOPHAGOSTOMUM DENTATUM (RUD.), A NEMATODE PARASITE OF THE PIG, WITH OBSERVATIONS ON THE STRUCTURE AND BIOLOGY OF THE FREE-LIVING LARVAE. Jour. Helminthol. 2 (1): 1-14, illus. 1924.

³ SCHWARTZ, B. GEOGRAPHICAL DISTRIBUTION OF OESOPHAGOSTOMUM LONGICAUDUM. (Note.) Jour. Parasitol. 12 (2): 113. 1925.

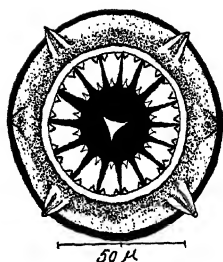


FIGURE 1.—*Oesophagostomum brevicaudum*; front view of head

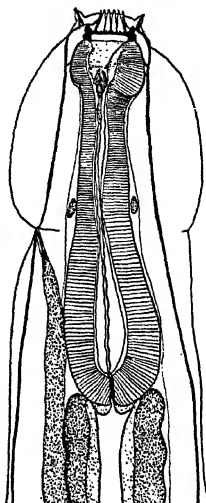


FIGURE 2.—*Oesophagostomum brevicaudum*; anterior portion of body. (Lateral view)

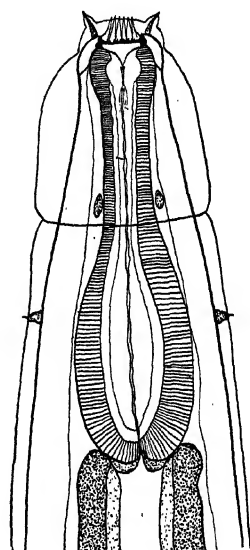


FIGURE 3.—*Oesophagostomum brevicaudum*; anterior view of body. (Ventral view)

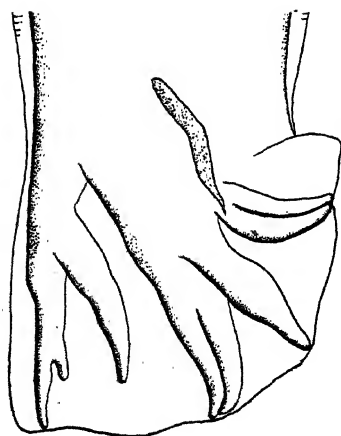


FIGURE 4.—*Oesophagostomum brevicaudum*; male bursa. (Lateral view)

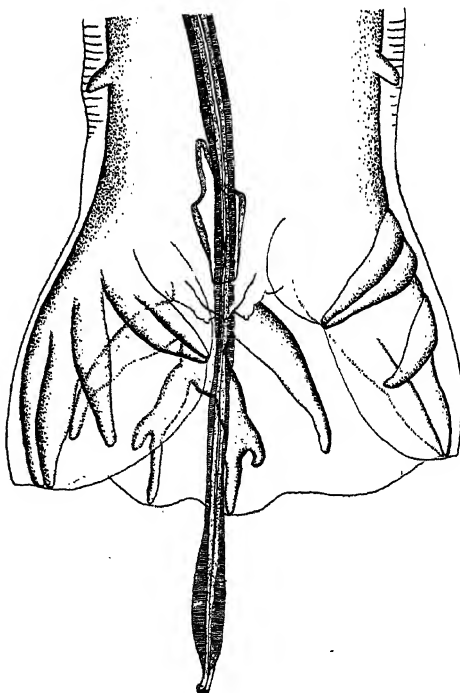


FIGURE 5.—*Oesophagostomum brevicaudum*; male bursa. (Ventral view)

groove behind is inflated. (Figs. 2 and 3.) The cervical groove is well developed ventrally and disappears dorsally. The cervical inflation of the cuticle extends to a point anterior to the middle of the esophagus. The esophagus, which is club shaped, has swollen sides at its beginning, and, as seen in lateral view, the dorsal wall of this swollen region is more developed than the corresponding ventral wall. (Figs. 2 and 12, A.) The inner wall of the swollen region contains a small toothlike protuberance on each side, projecting downward into the esophageal lumen. The outer margin of the cuticular lining of the esophagus is rounded into numerous knobs, replaced by a sinuous outline in some specimens. The excretory pore is immediately behind the cervical groove. The cervical papillae (fig. 3) are somewhat anterior to the last third of the esophagus.

Male.—According to observations of five specimens the variation in length is from 6.2 to 6.8 mm. and the range in maximum width is from 310μ to 449μ . The diameter of the body in the region of the prebursal papillae shows a variation of 173μ to 225μ . The esophagus is from 363μ to 449μ long by 124μ to 139μ in maximum width. The excretory pore is 171μ to 217μ from the beginning of the esophagus. The arrangement of the rays of the bursa (figs. 4 and 5) is similar to that of *Oesophagostomum dentatum* and *O. longicaudum*. The spicules are filiform, equal, and from 1.05 to 1.23 mm. long. The gubernaculum is from 98μ to 110μ long and from 37.5μ to 45μ in maximum width. The shapes of the gubernaculum and telamon (fig. 6) are similar to those described by Goodey for *O. dentatum*.

Female.—According to measurements of five specimens the variation in size is from 6.4 mm. to 8.5 mm. in length and from 310μ to 450μ in maximum width.

In the region of the vulva the diameter of the body varies from 108μ to 162μ and in the region of the anus it varies from 62μ to 70μ . The esophagus is from 434μ to 465μ long and from 120μ to 157μ in maximum width. The excretory pore is 186μ to 232μ from the beginning of the esophagus. The vulva, which is protuberant in some specimens, is 190μ to 225μ from the tip of the tail. The vagina (fig. 7) extends anteriorly and is from 218μ to 285μ long; the ojector apparatus is from 165μ to 195μ long. The eggs are from 52.5μ to 67.5μ long by 30μ to 45μ wide. The tail is from 81μ to 120μ long, is directed dorsad and ends in a slender tip.

Host.—*Sus scrofa domestica*.

Location.—Large intestine.

Locality.—Moultrie, Ga.

Type specimens (male and female).—Bureau of Animal Industry helminthological collections, United States National Museum No. 29038.

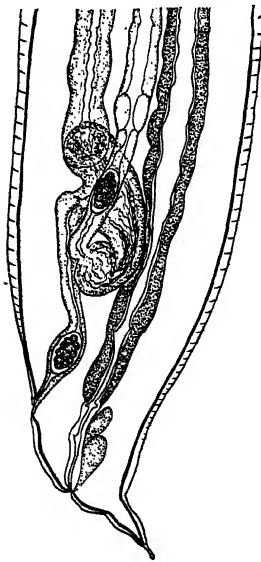
Paratypes.—United States Bureau of Animal Industry helminthological collections, United States National Museum No. 29039.

Oesophagostomum brevicaudum resembles *O. longicaudum* in the morphology of the esophagus and differs from the latter as well as from *O. dentatum* in many of the other specific characters. The principal differences are as follows: The walls of the buccal capsule in *O. brevicaudum*, as seen in optical section, present a characteristic appearance and differ from those of other species of *Oesophagostomum* parasitic in swine. (Fig. 12.) As seen in optical section (lateral view), the walls of *O. longicaudum* collected from swine in Georgia also show a distinct asymmetry, a character not noted by Goodey in his description of this species. Goodey's figure of the anterior view of this species shows symmetrical walls, diverging posteriorly. Aside from the relatively large



FIGURE 6.—*Oesophagostomum brevicaudum*: telamon and gubernaculum

50K



100K

FIGURE 7.—*Oesophagostomum brevicaudum*; posterior end of female. (Lateral view)

number of elements in the leaf crowns present in *O. brevicaudum* as compared with the number in *O. dentatum* and *O. longicaudum*, *O. brevicaudum* is readily recognized by the short female tail and the long vagina. The spicules in *O. brevicaudum* are considerably longer than those of *O. longicaudum* and are about the same length as those of *O. dentatum*.

***Oesophagostomum georgianum*, new species.**

The head structures are similar to those of *Oesophagostomum dentatum*. (Fig. 8.) The mouth is surrounded by an external leaf crown consisting of 9 elements and

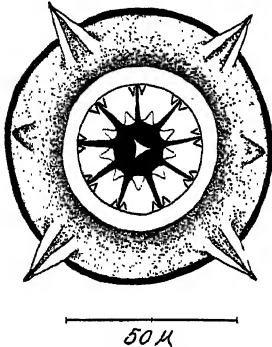


FIGURE 8.—*Oesophagostomum georgianum*; front view of head

an internal leaf crown of 18 elements, as in *O. dentatum* and in *O. longicaudum*. The esophagus is club-shaped, like that of *O. dentatum*.

Male.—The male is from 5.8 to 7.6 mm. long and from 388μ to 419μ in maximum width; the diameter of the body in the region of the prebursal papillae is from 232μ to 248μ . The excretory pore is immediately posterior to the cervical groove at a distance of 140μ to 186μ from the beginning of the esophagus. The esophagus is from 333μ to 377μ long and 109μ in maximum width. The width of the bursa when spread out is 310μ . The arrangement of the rays of the bursa (fig. 9) is similar to that shown in Goodey's figures⁴ of *Oesophagostomum dentatum* and *O. longicaudum*. The spicules are equal, filiform, and from 1.015 to 1.131 mm. long. The gubernaculum (fig. 10) is from 115μ to 130μ long, or somewhat longer, and differs from that of other species of the genus found in swine in that the anterior narrow portion of this structure is twisted, the entire structure resembling an inverted capital "L" when viewed from the side (fig. 9); in *O. dentatum*, *O. longicaudum*, and *O. brevicaudum*, the anterior portion of the gubernaculum is straight, and, when viewed from the side, it appears as an elongated body (fig. 4) with an indentation which forms an obtuse angle.

Female.—Of two specimens one was gravid. The mature female is 10 mm. long and 388μ in maximum width; the width of the body in the region of the vulva is 233μ and in the region of the anus, 93μ . The esophagus is 377μ long and 109μ in maximum width. The vulva (fig. 11) is 511μ from the tip of the tail; the tail is 217μ long and ends in a slender, terminal portion having a marked ventral curvature. The distance between the vulva and the anus is greater than the

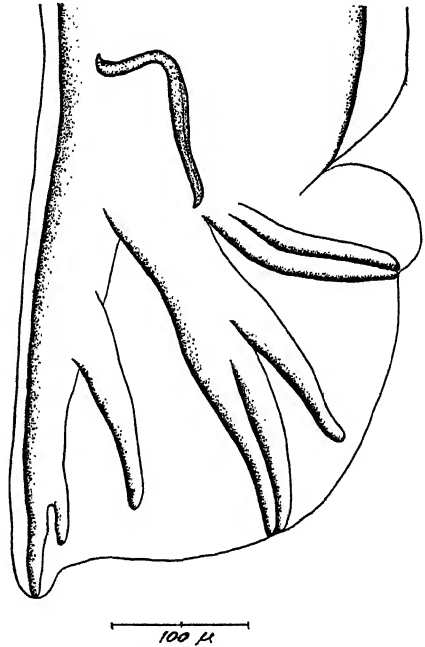


FIGURE 9.—*Oesophagostomum georgianum*; male bursa. (Lateral view)

⁴ GOODEY, T. *OESOPHAGOSTOMUM LONGICAUDUM* N. SP. FROM THE PIG IN NEW GUINEA. JOUR. HELMINTHOL. 3 (1): 45-50, illus. 1925.

length of the tail. In the mature specimen this distance is about 300μ , and in the immature specimen it is 217μ . The vagina is almost transversely elongated and is 109μ long in both specimens. The length of the ovejector apparatus in the mature specimen is 202μ , and in the immature specimen it is 155μ .

Practically all the measurements of the immature specimen are smaller than those of the gravid specimen. The length of the former is 5 mm. and its maximum width is 263μ . Its esophagus is 325μ long and 78μ in maximum width. The vulva in this specimen is 395μ from the tip of the tail. The tail is 178μ long.

Host.—*Sus scrofa domestica*.

Location.—Large intestine.

Locality.—Moultrie, Ga.

Type specimens (male and female).—Bureau of Animal Industry helminthological collection, United States National Museum No. 29064.

Paratypes.—Bureau of Animal Industry helminthological collection, United States National Museum No. 29065.

The heads of the four species of *Oesophagostomum* are shown in Figure 12.



FIGURE 10.—*Oesophagostomum georgianum*; gubernaculum

Oesophagostomum georgianum is very closely related to *O. dentatum*, differing from the latter in two important respects, namely, the shape of the gubernaculum and the shape of the female tail. As noted by Goodey, the gubernaculum in *O. dentatum* resembles a coal shovel or garden spade, with the handle and the blade about the same length. In *O. dentatum*, *O. longicaudum*, and *O. brevicaudum* the handle is straight, while in *O. georgianum*, it is distinctly twisted, as shown in Figure 10. In *O. georgianum* the handle is considerably shorter than the blade, whereas in *O. dentatum* these two parts are approximately equal. The female tail is longer in *O. dentatum* than in *O. georgianum*. The tail of the gravid female of the latter is 217μ long, whereas, according to Goodey, the length of the tail of *O. dentatum* is about 350μ in sexually mature forms. However, too much emphasis can not be placed, as a general rule, on the length of the female tail as a specific character in nematodes. Aside from differences in the length of the tail in the two species, an important difference in the shape of the tail has been noted. In this respect *O. georgianum* differs strikingly from *O. dentatum*; in the latter the female tail is straight and tapers to a point, whereas in *O. georgianum* the female tail is sinuous on its ventral surface and ends in a curved tip, the convexity of the curve being ventral and the terminal portion of the tail being directed dorsally. Another difference between *O. georgianum* and *O. dentatum* is the distance between the anus and the vulva as compared with the distance from the anus to the tip of the tail. In *O. dentatum* these two distances are approximately the

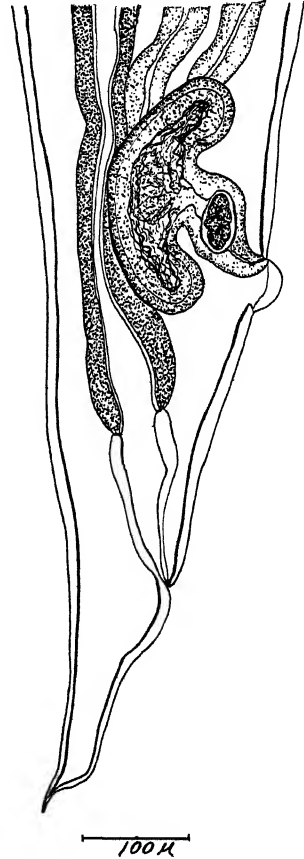


FIGURE 11.—*Oesophagostomum georgianum*; posterior end of female. Lateral view

same, the anus being equidistant from the vulva and the tip of the tail; in *O. georgianum* the distance between the tip of the tail and the anus is considerably smaller than that between the anus and the vulva.

Although the six specimens assigned to the species *Oesophagostomum georgianum* may perhaps represent aberrant forms of *O. dentatum*, it is believed that the differences between the forms which have been designated *O. georgianum* and *O. dentatum* warrant the recognition of the former as a distinct species. This view conforms to the usual custom

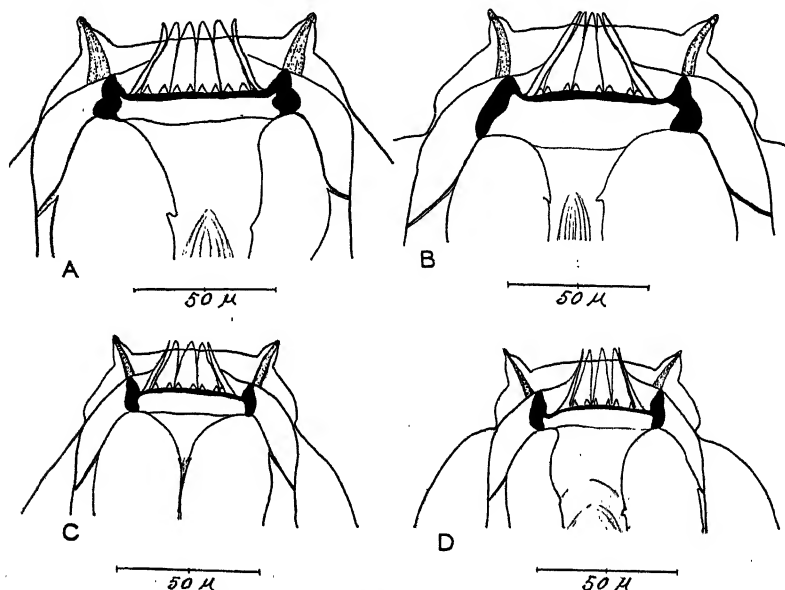


FIGURE 12.—A, Head of *Oesophagostomum brevicaudum*; B, head of *O. longicaudum*; C, head of *O. dentatum* D, head of *O. georgianum*

among helminthologists of creating new species in all cases in which the identity of closely related forms is doubtful.

SUMMARY

A morphological description is given of two new species of nodular worms (*Oesophagostomum*) recently found in the intestines of domestic swine. These species have been named *O. brevicaudum* and *O. georgianum* and, while the possibility has not been overlooked that the specimens herein assigned to the latter species may represent aberrant forms of *O. dentatum*, it is believed that observed morphological differences warrant the recognition of *O. georgianum* as a distinct species.

SELECTION CHARACTERS AS CORRELATED WITH PERCENTAGE OF SUCROSE, WEIGHT, AND SUCROSE CONTENT OF SUGAR BEETS¹

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INTRODUCTION

The purpose of this paper is to summarize briefly the available information on the selection characters of sugar beets; to correlate these and other selection characters of the beet with its percentage of sucrose, its weight, and its sucrose content under carefully controlled conditions; and to present certain new data that bear on this subject. Partial correlation coefficients are presented with special reference to the improvement of beets that are poor in yield or quality but which may be desirable from other viewpoints (for example, disease resistance). The studies reported may thus be considered as in a sense preliminary to the work of breeding sugar beets for better adaptation to the various areas of the United States.

The literature on the selection characters of the sugar beet is comprehensive (10)² and generally instructive, though certain conclusions are misleading, owing to the material used or the methods of investigation. This is rather to be expected, since some of this work marks the beginning of agricultural science. The literature deals almost entirely with the percentage of sucrose in relation to various selection characters, thus disregarding the weight and the sucrose content. With few exceptions, the investigators have used practical breeding material, which is unsuitable, according to Oetken (25), because breeders have previously eliminated all the material below the mean weight and rooty, large-crowned, and otherwise undesirable beets. This practical breeding material has been taken largely from mass selections rather than individual lines. Investigators have not controlled the water intake or outgo of beets, which necessarily influences the percentage of sucrose, in the beet roots (28). In many instances the data are questionable because the analyses of the beets were delayed until after storage (9, 26, 36). Only the more recent workers have used statistical methods for expressing these relationships among various characters. These are probably some of the reasons why this mass of literature contains many contradictory statements.

MATERIAL

The material studied consisted of five lots of sugar beets which had been grown previously at the United States Sugar Plant Field Station, Salt Lake City, Utah. Each lot was considered representative of

¹ Received for publication June 26, 1929; issued March, 1930.

² Reference is made by number (italic) to "Literature cited," p. 544.

the material used in sugar-beet breeding work. The material in its final form contained 496 beets. Two beets were injured during cultivation, and two were diseased. Except for this elimination the material was complete. No beets were discarded because of extreme weight, percentage of sucrose content, or any other character, except as herein stated. Because of the disadvantage of incomplete material for statistical purposes, the writer made an effort to retain the material in a form as nearly complete as possible.

The five lots of material were selected so as to represent lines of sugar beets in different stages of breeding development. Lots 2 and 3 were produced by individual selection, lots 1 and 4 from mass selection, and lot 5 is a European elite strain. When arranged in descending sequence with respect to the more important characteristics of the beet, these lots have the following order: In average percentage of sucrose—3, 2, 4, 1, 5; as to purity of juice—2, 3, 4, 1, 5; as to yield of topped beets—5, 2, 4, 1, 3; as to sucrose content—5, 2, 4, 3, 1; as to recoverable sugar—2, 5, 3, 4, 1. Lot 3 had the highest average percentage of sucrose, while lot 2 had the highest average purity of juice (44). Lots 2 and 3 are of the sugar type and lot 5 of the yield type. Lots 4 and 5 showed marked variation as to foliage characteristics. Lot 5 was the most vigorous, as is indicated by its foliage characteristics and superior yield.

METEOROLOGICAL DATA

The climatic conditions for the crop of this particular year (1925) were very favorable. Not only were the beets on the experimental plot good, but in general all other fields showed large yields. Utah and Idaho during this season produced their largest recorded acre yields of recoverable sugar. The meteorological data for the growing season are given in Table 1. These data show that the season of 1925 was characterized by greater precipitation and higher relative humidity than the normal season.

TABLE 1.—*Meteorological data for Salt Lake City, Utah, April 1 to October 31, 1925*

[Normal data for the same locality are shown for comparison]

Month	Season of 1925								Normal season							
	Temperature			Precipitation	Relative humidity			Sunshine	Temperature			Precipitation	Relative humidity			Sunshine
	Maximum	Mean	Minimum		6 a. m.	Noon	6 p. m.		Maximum	Mean	Minimum		6 a. m.	Noon	6 p. m.	
	° F.	° F.	° F.	In.	P. ct.	P. ct.	P. ct.	Hrs.	° F.	° F.	° F.	In.	P. ct.	P. ct.	P. ct.	Hrs.
April.....	60.9	51.7	42.5	2.37	58.9	41.0	38.7	93.2	59.5	49.6	39.8	2.04	60	47	40	* 83.8
May.....	74.0	63.3	52.6	1.90	52.4	31.6	30.2	345.7	68.5	57.8	47.1	1.99	57	36	35	300.8
June.....	75.8	65.6	55.3	2.80	59.5	39.3	35.2	302.8	79.3	67.4	55.5	.81	49	30	28	348.0
July.....	90.3	78.8	67.2	1.30	47.5	28.5	28.6	362.1	88.9	75.9	63.7	.55	46	30	25	362.1
August.....	83.8	73.0	62.2	1.65	53.0	30.5	29.3	303.5	86.5	74.6	62.6	.83	47	30	27	324.4
September.....	73.7	63.6	53.4	.29	58.6	35.1	33.0	276.8	76.9	64.5	52.8	.94	50	34	32	284.2
October.....	60.8	51.9	43.0	.65	57.3	37.8	41.8	230.5	62.6	52.3	42.1	1.52	59	43	46	230.5
Average.....	74.2	64.0	53.7	-----	55.3	34.8	33.8	-----	74.4	63.2	51.9	-----	52.6	35.7	33.3	-----
Total.....	-----	-----	-----	10.96	-----	-----	-----	1,914.6	-----	-----	-----	8.68	-----	-----	-----	1,933.8

* Only one-third of the sunshine for April is considered, because the plants did not appear above the ground until Apr. 20.

GENERAL METHODS

The five lots of seed were planted April 9, 1925, in five parallel rows on a plot of land which was selected because of its past record of uniformity in production. Soon after thinning, 100 consecutive beets from each row were given identification numbers that remained with them throughout the entire investigation. The soil, climate, and cultural conditions of these beets were very uniform, as the material was taken from relatively short sections at the middle of long rows.

During the last few years sugar-beet breeders have become increasingly aware of the need for measuring all sugar-beet characters in definite units. All but four characters herein given were measured in standard units or else compared with a standard.³ The work was carefully planned so that all measurements could be made on the same individual beets. The position of the beet in the soil and the foliage characters were determined by measurements in the field. As far as possible, the data were collected just before harvest; consequently the quality and quantity of the characters correspond very closely to the percentages of sucrose or to other valuable characteristics of the beets as determined at the time of analysis.

The procedure that made possible the accomplishment of the analysis before any considerable changes had occurred was as follows. At harvest the soil was removed from one side of each beet and the root was given its identification number by means of a permanent spiral tag (27) before it was removed from the ground. The leaves were trimmed off, leaving portions of the petioles about 5 mm. long on the crown. The leaves from each beet were weighed, and the beet was immediately placed in a covered can. As soon as the harvest had been completed the roots were cleaned, weighed to within 5 gm. of accuracy, and then analyzed. The percentage of sucrose (parts of sucrose per 100 parts of the total substance) in the beet was determined by direct polarization. The sucrose content (actual weight of sucrose) of the beet was determined by multiplying the weight of the root by its percentage of sucrose. The apparent purity was calculated from the percentage of sucrose in the juice and the total solids in the juice as determined by the refractometer. The other characteristics of the beet root were recorded soon after the chemical analysis. All beets were carefully protected against changes (28) during the entire period of harvest investigation. There were no beets that showed appreciable change of weight during this period, and therefore no corrections in the percentage of sucrose for water intake or outgo were necessary. The quantity of sugar loss due to other causes would have been negligible because of special care of the beets and their immediate analysis.

In all, enough measurements were made on this material for the calculation of about 3,000 correlation coefficients. From these data about 500 correlation coefficients have been calculated for the relationships between the valuable qualities of the beet root and the morphological, anatomical, physical, and chemical characteristics of the beet plant. The more valuable quantities of the beet root are its weight, its percentage of sucrose, its sucrose content, and its purity. The final criterion for evaluating the beet (the recoverable sugar per unit cost) is partly dependent upon these qualities.

³ The measurements of all characters were made by Vera Sanders and the writer.

The data for all lots were grouped, and the total correlation coefficients were calculated for the entire material. One disadvantage of this grouping is that a definite trend of relationship in one line may balance an equally definite reverse trend of relationship in a second line. To overcome this disadvantage the data for each line were kept separate and wherever a balancing of relationship between lines was noted correlation coefficients were calculated for the separate lines.

The data were classified and scatter diagrams were made for all characters. Sheppard's correction was made for grouping, and the correlation coefficients were calculated by the product-moment method. The biserial correlation coefficient was not used except for characters 7 and 11. The probable errors for the coefficients were calculated and read from Pearson's Table 7 (30). Partial and multiple correlation coefficients were calculated for the principal characters.

EXPERIMENTAL METHODS AND RESULTS

The total correlation coefficients for the relationships between the percentage of sucrose, the weight, and the sucrose content with 47 other characters of the beet plant for the entire material are given in Table 2. Table 3 gives partial correlation coefficients for some of these relationships.

The relationships among the various characters of the beet will be considered in the order given in Table 2. For convenience in referring to these characters and coefficients of correlation, it is necessary to indicate the characters by the numbers given in column 1 of Table 2. These numbers will also be used as correlation subscripts.

PERCENTAGE OF SUCROSE AND WEIGHT

CHARACTER 1.—The relationship between the percentage of sucrose in the root (character 1; sometimes erroneously designated as sugar content) and the weight of the beet root (character 2) is of major importance because it deals with the two fundamental characteristics of the sugar-beet root. This relationship has been studied and reported by many investigators. These authors differ in opinion as to the extent and existence of this relationship. Remy (37) summarizes the results of work covering seven years on 28 sorts, as follows: 17.44, 16.98, and 16.30 per cent of sucrose, corresponding to 331, 348, and 357 doppelzentners (220 pounds) yield of beets, respectively. These results indicate a negative relation between the percentage of sucrose and the weight of beet. Cetken's extensive work (25) shows both negative and positive relations between these two characters. Rümker (38) in his examination of 100,000 beets as to percentage of sucrose and weight found a negative relation between these characters, and states that with each 0.29 decrease in the percentage of sucrose there was an increase of 100 gm. in weight. Novotný (24) found that from 1892 to 1900 the percentage of sucrose decreased from 0.39 to 0.19 per cent for each 100 gm. of increased weight. Oetken (25) found that for certain lines the decrease in the percentage of sucrose was only 0.1 per cent per 100 gm. of the weight of beet. Fruwirth and others (10) state that the concept of a negative correlation between the percentage of sugar and the weight of beet should be dismissed and that these characters vary independently of each other.

TABLE 2.—Total correlation coefficients of the sugar-beet characteristics

[Descriptions of the various characters (numbered 1 to 50 in column 1) together with details of their measurement are given in correspondingly numbered paragraphs in the text]

No. and character	Coefficient of correlation with—		
	Percentage of sucrose	Weight	Sucrose content
1. Percentage of sucrose.....	—	—0.40±0.03	—0.23±0.03
2. Weight.....	—0.40±0.03	—	+ .95 ±. 00
3. Sugar content.....	— .23 ±. 03	+ .95 ±. 00	—
4. Angle of shoulder (obtuseness).....	— .32 ±. 03	+ .27 ±. 03	+ .25 ±. 03
5. Area of cross section of petiole.....	— .37 ±. 03	+ .34 ±. 03	+ .31 ±. 03
6. Area of leaf.....	— .33 ±. 03	+ .49 ±. 02	+ .46 ±. 02
7. Blonski selector.....	+ .23 ±. 03	— .25 ±. 03	— .22 ±. 03
8. Color of flesh.....	— .23 ±. 03	+ .35 ±. 03	+ .31 ±. 03
9. Color of leaf.....	+ .06 ±. 03	+ .25 ±. 03	+ .30 ±. 03
10. Conductivity.....	— .22 ±. 03	+ .28 ±. 03	+ .22 ±. 03
11. Density.....	+ .51 ±. 03	— .33 ±. 03	— .19 ±. 03
12. Density of foliage.....	— .21 ±. 03	+ .47 ±. 02	+ .66 ±. 02
13. Depth of petiole groove.....	— .14 ±. 03	+ .30 ±. 03	+ .28 ±. 03
14. Depth of root suture.....	+ .05 ±. 03	+ .05 ±. 03	+ .08 ±. 03
15. Diameter of crown.....	— .06 ±. 03	+ .86 ±. 01	+ .86 ±. 01
16. Dry substance in root.....	+ .81 ±. 01	— .30 ±. 03	— .15 ±. 03
17. Extension of shoulder.....	+ .29 ±. 03	— .10 ±. 03	— .06 ±. 03
18. Foliage area.....	— .35 ±. 03	+ .60 ±. 02	+ .58 ±. 02
19. Habit (prostrate foliage).....	+ .22 ±. 03	— .05 ±. 03	— .02 ±. 03
20. Hardness of root tissue.....	+ .43 ±. 02	— .26 ±. 03	— .19 ±. 03
21. Height of crown.....	— .46 ±. 02	+ .77 ±. 01	+ .74 ±. 01
22. Leaf density.....	+ .37 ±. 03	— .64 ±. 02	— .04 ±. 02
23. Length of leaf.....	— .32 ±. 03	+ .38 ±. 03	+ .41 ±. 02
24. Length of root.....	— .14 ±. 03	+ .75 ±. 01	+ .64 ±. 02
25. Margin of leaf.....	— .19 ±. 03	+ .17 ±. 03	+ .16 ±. 03
26. Number of leaves.....	— .22 ±. 03	+ .54 ±. 02	+ .62 ±. 02
27. Number of vascular-bundle rings.....	— .01 ±. 03	+ .36 ±. 03	+ .39 ±. 03
28. Position in the soil.....	— .30 ±. 03	+ .70 ±. 02	+ .69 ±. 02
29. Purity (apparent).....	+ .38 ±. 03	— .17 ±. 03	— .10 ±. 03
30. Refractometer reading.....	+ .81 ±. 01	— .30 ±. 03	— .15 ±. 03
31. Relative depth of petiole groove.....	+ .00 ±. 03	+ .13 ±. 03	+ .16 ±. 03
32. Relative flatness of root.....	+ .05 ±. 03	+ .03 ±. 03	+ .04 ±. 03
33. Relative height of foliage.....	— .16 ±. 03	+ .10 ±. 03	+ .02 ±. 03
34. Relative length of blade.....	— .07 ±. 03	— .03 ±. 03	— .05 ±. 03
35. Relative length of leaf.....	— .03 ±. 03	— .09 ±. 03	— .10 ±. 03
36. Relative length of petiole.....	+ .07 ±. 03	— .10 ±. 03	— .10 ±. 03
37. Relative length of root.....	+ .14 ±. 03	— .19 ±. 03	— .20 ±. 03
38. Relative space per beet.....	— .02 ±. 03	+ .20 ±. 03	+ .21 ±. 03
39. Ring density.....	+ .31 ±. 03	— .68 ±. 02	— .67 ±. 02
40. Rootiness of beet.....	— .17 ±. 03	+ .12 ±. 03	+ .11 ±. 03
41. Roughness of skin.....	+ .36 ±. 03	+ .01 ±. 03	+ .08 ±. 03
42. Rugosity of leaf surface.....	— .17 ±. 03	+ .16 ±. 03	+ .18 ±. 03
43. Shade of leaf.....	+ .24 ±. 03	+ .10 ±. 03	+ .17 ±. 03
44. Sugar marks.....	+ .17 ±. 03	+ .12 ±. 03	+ .16 ±. 03
45. Thickness of leaf.....	— .14 ±. 03	+ .17 ±. 03	+ .16 ±. 03
46. Turn of root suture.....	+ .03 ±. 03	— .05 ±. 03	— .04 ±. 03
47. Weight of foliage.....	— .33 ±. 03	+ .56 ±. 02	+ .54 ±. 02
48. Width of leaf.....	— .23 ±. 03	+ .41 ±. 03	+ .43 ±. 02
49. Width of petiole groove.....	— .25 ±. 03	+ .23 ±. 03	+ .20 ±. 03
50. Width of root suture.....	— .10 ±. 03	+ .02 ±. 03	— .00 ±. 03

Table 2 shows a negative correlation coefficient (-0.40 ± 0.03) between the percentage of sucrose and the weight of beet for all the material investigated. For this relationship the separate lines gave the following values of r : 1, -0.27 ± 0.06 ; 2, -0.52 ± 0.05 ; 3, -0.33 ± 0.06 ; 4, -0.53 ± 0.05 ; and 5, -0.48 ± 0.06 . These coefficients are quite different for the different individual lines, yet they are of the same sign. As would be expected, the correlation coefficients are less for the more homogeneous lines. Since Velasco (45) states that the leaves and the weight of the beet are functions of climate and conditions, it might be asked are these relationships between the percentage of sucrose and the weight of beet controlled by these factors? So far as climate is concerned, it must have been uniform over this small experimental plot. The available space for each beet in the field was

different, but this experimental difficulty was overcome by calculating the partial correlation coefficients. As the partial correlation coefficient for the percentage of sucrose with the weight constant for relative space ($r_{12.38} = -0.4036 \pm 0.03$) is practically identical with the total correlation coefficient ($r_{12} = -0.40 \pm 0.03$), there is no indication that the relationship between the characters 1 and 2 is affected by the available space in the field. Even though available space does not influence this relationship, it has a direct effect upon character 2, as will be pointed out later. The partial correlation coefficient of the percentage of sucrose with the weight constant for sucrose content of the beet root was calculated as -0.6271 ± 0.02 , indicating that this relationship between characters 1 and 2 is influenced by the sucrose content of the root. These results with the majority of investigations and the usual field and factory results in all sugar-beet countries show that there is in general a negative relation between the percentage of sucrose in the beet root and its weight. This relationship has a physiological explanation and should for the present be spoken of as physiological correlation. It is not possible from the data now available to ascertain what part, if any, of this relationship is hereditary in nature, or whether there is any true correlation between characters 1 and 2.

PERCENTAGE OF SUCROSE AND SUCROSE CONTENT

CHARACTER 2.—The relationship between the percentage of sucrose in the beet root (character 1) and the sucrose content of the beet root (actual quantity of sucrose, character 3) is of importance because 3 approaches the criterion for evaluating sugar beets. Janasz (12), in comparing various sugar-beet races, records figures for characters 1 and 3. Plahn (33) and Mass (17) state that character 1 decreased whereas character 3 increased. The total correlation coefficient for the relationship between characters 1 and 3 is given in Table 2 as -0.23 ± 0.03 . The separate lines gave the following total correlation coefficients for this relationship: 1, -0.14 ± 0.07 ; 2, -0.41 ± 0.06 ; 3, -0.24 ± 0.07 ; 4, -0.24 ± 0.07 ; and 5, -0.27 ± 0.07 . It was thought that the sucrose content (character 3) should increase with the increase of percentage of sucrose in the root (character 1), but these coefficients signify a negative relation. From this it would appear that beets of the highest sucrose content would be bred by selecting those of low percentage of sucrose. The inconsistency of this was apparent, and the first step toward clarifying the situation was to determine the partial correlation coefficient $r_{13.2}$. The value for this partial correlation of character 1 with character 3 constant for character 2 was calculated as $+0.52 \pm 0.02$. (Table 3.) This coefficient is significant, and shows that the sucrose content does increase as the percentage of sucrose increases so long as the beets are of uniform weight. This explains why earlier investigators reported a negative relation between characters 1 and 3, instead of the positive relationship which exists, and shows the inconsistency of selecting low percentage sugar beets for high sucrose content. As these results show that the percentage of sucrose is positively correlated with the sucrose content when the factor for root weight is eliminated, the increase of the percentage of sucrose in the beet is of primary importance to sugar-beet breeding. It may be added that the first and second order

coefficients of correlation $r_{13-38} = -0.23 \pm 0.03$ and $r_{13-38\ 2} = +0.52 \pm 0.02$ are the same as the total and first order coefficients $r_{13} = -0.23 \pm 0.03$ and $r_{13-2} = +0.52 \pm 0.02$, indicating that available space in the field does not influence the relation between characters 1 and 3.

TABLE 3.—Partial correlation coefficients of sugar-beet characters

Selection character No.	Characters of column 1 correlated with—				Selection character No.	Characters of column 1 correlated with—			
	Percentage of sucrose, constant for weight	Weight constant for percentage of sucrose	Sucrose content, constant for percentage of sucrose	Sucrose content, constant for weight		Percentage of sucrose, constant for weight	Weight constant for percentage of sucrose	Sucrose content, constant for percentage of sucrose	Sucrose content, constant for weight
1	2	3	4	5	1	2	3	4	5
1				+0.52	22	+0.16	-0.58	-0.61	-0.13
2			+0.96		23	-0.19	+0.29	+0.37	+0.17
3	+0.52	+0.96			24	+0.26	+0.76	+0.63	-0.29
4	-0.25	+0.16	+0.19	-0.02	25	-0.14	+0.10	+0.12	-0.01
5	-0.26	+0.23	+0.25	-0.04	26	-0.00	+0.67	+0.51	+0.00
6	-0.17	+0.34	+0.42	-0.02	27	+0.16	+0.39	+0.40	+0.12
7	+0.15	-0.18	-0.18	+0.06	28	-0.17	+0.64	+0.67	+0.11
8	-0.10	+0.29	+0.27	-0.08	29	+0.35	-0.02	-0.01	+0.20
9	+0.18	+0.30	+0.32	+0.21	30	+0.79	+0.04	+0.06	+0.42
10	-0.12	+0.21	+0.18	-0.15	38	+0.06	+0.21	+0.21	+0.07
11	+0.44	-0.16	-0.09	+0.42	39	+0.06	-0.64	-0.65	-0.10
13	-0.02	+0.27	+0.26	-0.06	41	+0.40	+0.18	+0.18	+0.22
15	-0.03	+0.72	+0.86	+0.27	42	-0.12	+0.10	+0.15	+0.09
16	+0.79	+0.04	+0.06	+0.45	43	+0.31	+0.21	+0.24	+0.24
17	+0.27	+0.02	+0.01	+0.11	44	+0.13	+0.21	+0.21	+0.15
18	-0.15	+0.54	+0.55	+0.04	45	-0.08	+0.13	+0.13	-0.01
20	+0.37	-0.11	-0.10	+0.19	48	-0.08	+0.36	+0.40	+0.14
21	-0.28	+0.72	+0.73	+0.04	49	-0.18	+0.15	+0.15	-0.06

WEIGHT AND SUCROSE CONTENT

CHARACTER 3.—The relationship between the weight of the beet root (character 2) and the sucrose content of the beet root (character 3) is of great significance and has been seriously neglected by sugar-beet breeders. Janasz (12) published three figures indicating a positive relation between characters 2 and 3. Mass (17) showed that as the weight of feed beets increased their sugar content increased. Pritchard (35) published the first statistics expressing the relationship between characters 2 and 3 as $+0.92 \pm 0.0016$. Table 2 gives the total correlation coefficient for this relationship as $+0.95 \pm 0.00$. For this relationship the separate lines gave the following values for the total correlation coefficients: 1, $+0.98 \pm 0.01$; 2, $+0.98 \pm 0.01$; 3, $+0.94 \pm 0.01$; 4, $+0.98 \pm 0.01$; and 5, $+0.96 \pm 0.01$. The following partial correlation coefficients $r_{23-38} = +0.95 \pm 0.00$, $r_{23-1} = +0.96 \pm 0.00$, and $r_{23-38\ 1} = +0.96 \pm 0.00$ show that this relationship is not seriously influenced by either character 1 or character 38. All of these coefficients are significant and their values show a very strong relationship between characters 2 and 3. It is at once apparent that any gain in the weight of the beet will be accompanied by a corresponding increase in the sucrose content. In view of the fact that the sucrose content of the beet is directly proportional to the yield of sugar within certain minor limitations as has been noted, the sugar-beet breeder should give the weight of the root more consideration than has been done in the past.

PERCENTAGE OF SUCROSE WEIGHT AND SUCROSE CONTENT AS RELATED TO OTHER SELECTION CHARACTERS

The methods used in measuring each sugar-beet characteristic for all individual beets and in collecting the data for the correlations will be briefly stated. All of the typical relationships will be considered individually. The total correlations describing all relations for the entire material are presented in Table 2. Note will be made of any total correlation for the individual lines and the means of the individual lines that vary widely from those given in Table 2.

CHARACTER 4.—The angle which is formed by the intersection of the crown and root body surfaces was referred to in Table 2 as the "angle of shoulder." This angle was measured by a protractor. The coefficients $r_{14} = -0.32 \pm 0.03$ (Table 2) and $r_{14.2} = -0.25$ (Table 3) show that beets with the more obtuse shoulder angles generally have a lower percentage of sucrose, or the correlative, that beets with more acute shoulder angles generally have a higher percentage of sucrose. Yet, the values for $r_{24} = +0.27 \pm 0.03$ and $r_{34} = +0.25 \pm 0.03$ indicate that beets having the more obtuse shoulder angles are larger and contain more sucrose. The partial coefficients $r_{24.1} = +0.16$ and $r_{34.1} = +0.19$ support these conclusions even after the relations with character 1 have been eliminated. The partial coefficient $r_{34.1} = +0.19$ is significant, whereas the partial coefficient $r_{34.2} = -0.02$ is not significant. This indicates that the larger sucrose content of beets with obtuse shoulder angles is essentially due to the larger weight of this type of beet root. Similar relations existed for the individual lines, but they were of irregular magnitude.

CHARACTER 5.—The area of the cross section of the sugar-beet petiole, aside from individual-plant differences, varies with the age and size of the leaf, the seasonal and field conditions, and the particular time of the grand period of growth in which the petiole was produced; thus, the first and last petioles produced by the beet are small. Finally the cross-section area of any beet petiole increases in the proximity of the leaf blade and the root crown. To avoid these and other difficulties, a certain leaf on each beet was selected for study. This leaf was termed by the writer the type leaf. The length of its leaf blade when near its height of vigor, growth, and development was 0.7 that of the longest leaf blades on the particular plant and specially characteristic of the plant. At harvest the petiole of the type leaf was sectioned with a razor at the point of minimum area and an ink stamp of its area recorded. The areas of these stamps were later determined by a planimeter and expressed in square millimeters. The correlation coefficients for character 5 with characters 1, 2, and 3 are similar to those for character 4 (Tables 2 and 3) and will permit of a like analysis with similar conclusions. (See character 4.)

CHARACTER 6.—Corenwinder and Contamine (6) were of the opinion that large leaves indicate a high percentage of sucrose. Vychinski (48) states that beets grown in the border row have large leaves and low percentages of sucrose. Kajanus (13) notes that large beets have large leaves, while Knauer and Doerstling (14) and Doerstling (?) prefer an average-sized leaf. Kneifel (15) and Marek (16) state that the size of the leaf varies throughout the season and with various types of soil. Investigators who have studied the total leaf surface of beets are mentioned under character 18. The type-leaf blade of each beet was photographed at harvest and its area measured as

square centimeters by a planimeter. The correlative coefficients for character 6 are similar to those for characters 4 and 5 (Tables 2 and 3) and will permit of like conclusions. (See character 4.)

CHARACTER 7.—Blonski (1) noted that beets having high percentages of sucrose were long in proportion to their diameter and he devised the Blonski selector for eliminating undesirable beets. Briem (3) criticized Blonski and stated that beets having a high percentage of sucrose may be either long or short. A selector was constructed by the writer according to Blonski's directions and each beet tested. Even though there are irregularities, as noted by Briem, the results in Tables 2 and 3 indicate a positive correlation between characters 1 and 7, as was first noted by Blonski.

CHARACTER 8.—The color of the beet flesh was determined by the writer from a sample located midway between the center and periphery of a middle cross section of the beet body. The following five colors illustrated by Ridgway⁴ were recognized: 29'' g, 26'' g, white pallid-neutral gray, 20'' g, and white. In general, all beets were white, there being no deeply colored individuals, as spoken of by Munerati (21). Beets with even these very slight flesh colors tend toward a lower percentage of sucrose but a higher weight and sucrose content.

CHARACTER 9.—Some investigators noted increased percentage of sugar with dark-green foliage, others noted the opposite relation, and still others noted no relation. These varying opinions are due in part to the fact that the color of the sugar-beet leaf varies with age, light exposure, moisture, soil nutrients, and the season of the year. These difficulties were avoided by taking a sample from a particular locality on the type leaf. This sample was compared with a chart of standards distinguishing 20 different hues of green. These colors were made particularly for sugar-beet foliage, and range from Ridgway's 26''' m to his lighter green 26 i. The total correlation coefficients of Table 2 show that character 9 is negligibly related to character 1, but positively related with characters 2 and 3. Late-maturing beets retain their dark-green foliage longer in the season. As these beets are usually large, it was possible to eliminate the error due to late-maturing beets by keeping the weight of the beets constant. This partial correlation of characters 1 and 9 constant for $2(r_{19.2} = +0.18 \pm 0.03)$ is positive, and suggests a slight relation between the percentage of sucrose and the dark-colored foliage. It should be noted that the partial coefficients for character 9 with characters 1, 2, and 3 are all positive and significant. (Table 3.) This unique association shows that the percentage of sucrose, the weight, and the sucrose content of the sugar-beet root increase with the dark-green color of the foliage. Breeders should scrutinize this character in the improvement of sugar beets.

CHARACTER 10.—Conductivity was measured by inserting two platinized knife blades into the beet tissue at a definite point (halfway between the fleshy and suture sides and one-third the distance from the crown to the tip of the beet). In general, a lower conductivity was noted at the points of higher concentration of sucrose in the individual beet. This agrees with the work of Colin and Grandsire (5), though they worked on the extracted juice. The total and partial correlations for all the material and the individual lines showed

⁴ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

negative relation between characters 1 and 10, but positive relations between 2 and 10 and between 3 and 10.

CHARACTER 11.—The relation between the density of the beet and its percentage of sucrose has been studied by many writers. The beets were divided into two groups, "sinkers" and "floaters," by submerging a piece of tissue, as previously outlined by the writer (29), in a salt solution. The coefficients in Table 2 show that density is positively correlated with the percentage of sucrose, but negatively related to the weight and the sucrose content of the beet. These negative relations for character 11 with characters 2 and 3 were reduced and became less significant as the influence of character 1 was eliminated. The value of this relationship between density and the percentage of sucrose increased for the individual lines and amounted in one case to +0.74. This was undoubtedly due to the fact that the beets of pure lines are more uniform than the beets of heterogeneous lots in structure and in content of gases and salts—the characteristics that interfere with the density and the percentage of sucrose relationships. For a more complete analysis of the coefficients, see the paragraph relating to character 16, wherein a similar set of relationships is described.

CHARACTER 12.—The density of the foliage was estimated during September. It was noticed that this factor changed with the different periods of beet development and the changing external conditions. Owing to these and other difficulties (see character 22), the relations between density of foliage and other characters are questionable.

CHARACTER 13.—Pritchard (35) found a slight positive correlation between percentage of sucrose and depth of petiole groove. From the stamps of the petiole cross sections, made as described under character 5, the depths of the petiole grooves were accurately measured in millimeters. The total coefficients $r_{1,13}$ of Table 2 indicate a negative relation between characters 1 and 13. The partial correlation coefficient ($r_{1,13,2} = -0.02 \pm 0.03$) shows that there was no association of character 1 with character 13 if the weight of the beet was held constant. As expected, the depth of the petiole groove increases with the weight and the sucrose content of beets. (Tables 2 and 3.)

CHARACTER 14.—A deep suture on the root has associated with a high percentage of sucrose. In this connection the beet in the time of Achar'd had no well-defined suture. The depth of the suture was estimated by comparison with its width and should be accepted with caution. The correlation coefficients for character 14, given in Table 2, are not significant.

CHARACTER 15.—Beets with small crowns usually have the higher percentages of sucrose. The diameter of the crown was accurately measured with calipers at the point of the first leaf scar. The total coefficients of Table 2 indicate that characters 2 and 3 increased with character 15, whereas character 1 decreased. The partial coefficients of Table 3 show definitely that the percentage of sucrose was not significantly correlated with the diameter of the beet crown and that beets with large crowns were large and high in sucrose content. The sucrose content is significantly correlated with diameter of crown regardless of whether either character 1 or 2 was held constant.

CHARACTER 16.—It is generally conceded that dry substance is positively correlated with the percentage of sucrose. The dry substance in the beet root was determined by the refractometer. The total

and partial coefficients of Tables 2 and 3 show that the percentage of sucrose is significantly and positively correlated with dry substance. The total correlation coefficients of Table 2 indicate that the weight and the sucrose content are negatively correlated with dry substance. These negative relations are only apparent and are due to the strong positive relation between characters 1 and 16. The partial coefficients $r_{2\ 16-1} = +0.04$ and $r_{3\ 16-1} = +0.06$ of Table 3 show that the weight and the sucrose content are not significantly associated with dry substance if the factor for the percentage of sucrose is held constant. The significant coefficient $r_{3\ 16-2} = +0.42$ is due to the factor for percentage of sucrose not being eliminated.

CHARACTER 17.—The maximum extension of the surface of the beet beyond the first leaf scar was termed the extension of the shoulder. The percentage of sucrose increases with the extension of the shoulder, but there is no significant relation between character 17 and characters 2 and 3. The relations between characters 17 and 1 were very different for the individual lines, ranging from +0.17 to +0.40. However, the lines with the highest mean percentage of sucrose always had the largest mean extension of shoulder. Although the partial coefficients for character 17 are not so significant as those for character 16 (Table 3), similar conclusions should be drawn. (See character 16.)

CHARACTER 18.—The areas of beet leaves have been variously measured or estimated by several writers. Mass (17) found no relation between the leaf area and the percentage of sucrose. Munerati and his coworkers (23) found that defoliation of beets caused a decreased percentage of sucrose. Corenwinder and Contamine (6) claimed positive relation between characters 1 and 18, but Vychinski (48) questioned this. The total leaf surface of the beet during its entire vegetative period was in the present study termed the "foliage area." So far as the writer knows, this is the first attempt that has been made to determine the total foliage area produced by a beet. After planimeter measurements had been made of the total leaf area of beets during three different years it was found that the foliage area of a beet plant could be estimated by multiplying the area of the type leaf (character 5) by the total number of leaves produced during the vegetative period (character 26). The total correlation coefficients in Table 2 show that an increase of foliage area is accompanied by decreased percentage of sucrose, increased weight, and increased sucrose content. The partial correlation of the percentage of sucrose (character 1) with the foliage area (character 18) constant for the weight (character 2) gives a coefficient that is still significant ($r_{1\ 18-2} = -0.15 \pm 0.03$). Finally, the coefficient $r_{1\ 18-23} = -0.21 \pm 0.03$ shows that characters 1 and 18 are negatively correlated after the factors of weight and of sucrose content are eliminated. It was hoped to establish positive net relation between characters 1 and 18, but so far as the other factors have been considered the reverse is true; i. e., the percentage of sucrose increases with the decrease of foliage area. All the total and partial correlation coefficients show that the weight of the beet increases directly with the foliage area. The following coefficients— $r_{3\ 18} = +0.58 \pm 0.02$, $r_{3\ 18-1} = +0.55 \pm 0.02$, and $r_{3\ 18-12} = +0.15 \pm 0.03$ —show that the sucrose content increases with the foliage area.

CHARACTER 19.—Earlier investigators, with few exceptions, have noted an increased percentage of sucrose in the prostrate-foliage type of beet. The description prostrate-foliage habit was used to describe the angle that the leaf makes with the perpendicular. This angle is smaller for the younger leaves and increases with the age of the leaf. Leaf maturity, excessive transpiration (due to drought, high temperature, wind, dry soil, etc.), a lack of phosphorus, and hereditary characteristics are the more important causes of the prostrate habit. Owing to these disturbing factors, the type leaf was examined during the same period of the day and under as nearly uniform conditions as possible. Table 2 shows that character 19 is positively related to character 1 but not related to either character 2 or 3.

CHARACTER 20.—According to Geschwind (11), De Varies and Stoklasa found that lignified beet contains a smaller percentage of sucrose. Munerati (20) states that woody beets are not necessarily the bolting strains. Schribaux (41) states that the flesh of sugar beets has become firm and harder with the breeding of beets of increased percentage of sucrose. He states that Pelletier determined the value of beets by the force required to push a punch into the tissue. The hardness of the beet tissue was determined by a self-recording apparatus devised particularly for this type of work. There is no question but that beets of an average higher percentage of sucrose can be selected by their firmness ($r_{1\ 20} = +0.43 \pm 0.02$ and $r_{1\ 20.2} = +0.37$). It is easy to distinguish the excessively woody beets referred to by De Varies and Stoklasa by their extreme hardness. Under character 16 a similar set of partial correlation coefficients is described.

CHARACTER 21.—Various investigators have noted that the flat-crowned beets were of a higher percentage of sucrose. The height of the crown was measured in centimeters and represents the vertical distance from the first leaf scar to the apex of the crown. The relations of character 21 with characters 1, 2, and 3 are very significant. These coefficients (Tables 2 and 3) are similar to those for character 4 and should be similarly analyzed. (See character 4.)

CHARACTER 22.—In making estimates of the density of foliage (character 12) the estimate is always influenced by the size of the leaf as well as the number of leaves. Therefore, the density of foliage of beets with large leaves is overestimated, and this explains the negative relation between the density of foliage (character 12) and the percentage of sucrose. (See also correlation coefficient for character 6 and 1.) For this reason the writer devised the term "leaf density," which describes the ratio of the number of leaves on one parastichy (character 26) to the distance from the first leaf scar to the apex of the crown measured in centimeters. This ratio for the density of leaves on the sugar-beet crown corresponds exactly to the "ring density" of Seeliger (42). The leaf-density ratio describes the leaf density accurately. The leaf density is positively correlated with the percentage of sucrose, but negatively correlated with the weight and the sucrose content. This is consistent with the coefficients $r_{1\ 39}$, $r_{2\ 39}$, $r_{3\ 39}$ and with all other coefficients dealing with the number of leaves, the number of vascular-bundle rings, the diameter of crown, and the diameter of root. The leaf density increases with the percentage of sucrose, as is shown by the total and partial correlation coefficients for the entire material, all individual lines, and the means of individual

lines. The significant negative coefficients $r_{2\ 22.1}$ and $r_{3\ 22.1}$ indicate a strong association of characters 2 and 3 with character 22. In this particular instance the total coefficients $r_{1\ 22}$ and $r_{3\ 22}$ are more reliable than the partial coefficient $r_{1\ 22.2}$ and $r_{3\ 22.2}$ because the diameter of the root (which corresponds very closely to the weight) was considered in the original data (definition of leaf density), and the weight should not be held constant.

CHARACTER 23.—It was thought that beets having high percentages of sucrose had long leaves, though the results of Pritchard (35) indicate the reverse relation. The length of the leaf blade was judged by the length of the type leaf blade measured in centimeters. The coefficients of Tables 2 and 3 show that the length of the leaf blade was negatively correlated with the percentage of sucrose and positively with the weight and the sucrose content. The partial coefficients $r_{3\ 23.1}$ and $r_{3\ 23.2}$ show that sucrose content is significantly correlated with the length of leaf regardless of whether either character 1 or 2 was held constant.

CHARACTER 24.—Some investigators have noted a positive relation between the percentage of sucrose and the length of beet root, but others have noted no such relation. Florian (8) states that short beets have a higher percentage of sucrose. Briem (4) noted that the weight of the beet was directly related to the length of the root. The length of each root was expressed in centimeters. Table 2 shows that long beets have a slightly smaller percentage of sucrose but are heavier and have greater sucrose content. The peculiar character of the partial coefficients in Table 3 is due to the significant relations between the weight and the absolute length of root, and to the fact that holding the weight constant transforms the data to a relative basis. (See character 37.)

CHARACTER 25.—Plot (34) noted that beets of high percentages of sucrose had leaves with undulated margins, while Kneifel (15) and Pritchard (35) found no relation between the percentage of sucrose and the character of leaf margin. The margin of the leaf was described as to its undulated or flexuose character. The higher values were given for the more undulated margins. As the undulated characters of the margin vary with the age of the leaf, the type leaf was considered characteristic of each plant. It appears that beets having plain or straight-margined leaves have a slightly higher percentage of sucrose.

CHARACTER 26.—The number of leaves was found to be positively related to the percentage of sucrose by certain writers and negatively related by others. Sengbusch (43) found no difference in the number of leaves produced by the four brands of Klein Wanzleben beets (ZZ, Z, N, and E), although they show marked differences as to the percentage of sucrose. Vychinski (48) states that beets of high percentages of sucrose have few leaves. The earlier investigators usually considered the number of leaves on the beet at harvest. As the first leaves fall off during the summer, the leaf count at any one particular time would not represent the total number of leaves produced by the plant. For this work the leaves on each beet were counted in June, August, and October, so that the numbers represent the total number of leaves produced during the entire vegetative period. The writer noted in 1923 that the leaves of beets (sugar

and feed types) were arranged along five prominent parastichies, which extend spirally from the first five leaf scars to the crown apex. Beets are of two types: (1) Those having the clockwise and (2) those having the counterclockwise parastichies. (Fig. 1.) It was found that the total number of leaves produced by any beet could be accurately and quickly determined by simply counting the average number of leaves and leaf scars per parastichy and multiplying by 5. This method checked very closely with the individual leaf counts made during June, August, and October. The correlation coefficients of Table 2 for character 26 with characters 1, 2, and 3 are significant. The partial coefficients of Table 3 indicate that the percentage of sucrose is not significantly correlated with the number of leaves, but

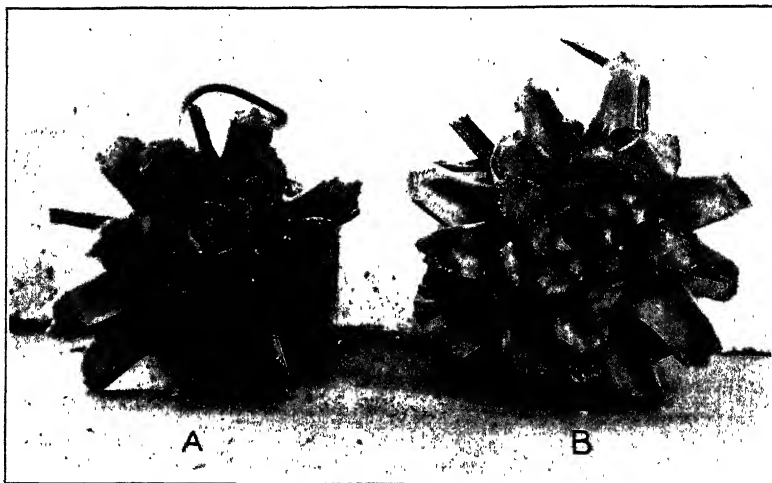


FIGURE 1.—A, Arrangement of leaves on a beet having clockwise parastichies and counterclockwise genetic spiral; B, arrangement of leaves on a beet having counterclockwise parastichies and clockwise genetic spiral

that the weight and the sucrose content are positively and significantly correlated with the number of leaves.

CHARACTER 27.—Certain investigators have noted a higher percentage of sucrose in beets having many vascular-bundle rings. Sengbusch (43) noted no difference between the four brands, ZZ, Z, N, and E as to number of rings. Pitsch (32) and Schneider (40) questioned the importance of ring numbers. The number of vascular bundle rings was in this instance counted with a hand lens from thin sections. The sections were cut from an intermediate point between the fleshy and suture surfaces and at the largest diameter of the beet body. The total correlation coefficients show no significant relation between characters 1 and 27. However, the partial correlation coefficient $r_{1\ 27.2} = +0.16 \pm 0.03$ shows that with beets of constant weight the percentage of sucrose increases slightly with the number of vascular bundle rings. This character is positively correlated with the percentage of sucrose, the weight, and the sucrose content. (See character 9.)

CHARACTER 28.—It is generally conceded that beets protruding high above the ground are low in percentage of sucrose. Vilmorin

(46) casts some doubt on this relation, and Saillard (39) noted that beets grew high out of the ground on certain types of soil with no resulting difference in the percentage of sucrose. Meyer (19) advocated the breeding of round protruding beets which carry less dirt and are easily dug even though the percentage of sucrose is slightly less. The position in the soil of each beet was measured in centimeters and represents the vertical distance from the soil level to the crown apex. The coefficients are similar to those for character 23. (See character 23.)

CHARACTER 29.—The purity coefficient is in general directly proportional to the percentage of sucrose. Urban (44) and Sengbusch (43) noted that some lines with lower percentages of sucrose actually had higher purity. The apparent purity was determined from refractometer reading and the percentage of sucrose. The coefficients for character 29 are very similar to those for character 16 and will admit of like analysis. (See character 16.)

CHARACTER 30.—The percentage of sucrose is positively correlated with the dry substance in the beet (see character 16) simply because the principal part of the dry substance is sucrose. The density of a sugar solution increases as its percentage of dry substance increases. Finally, the index of refraction is proportional to the density of the solution, and the refractometer reading is positively correlated with the percentage of sucrose. Many of the investigators cited under characters 11 and 16 contributed to the use of the refractometer for estimating the percentage of sucrose. The refractometer readings were either high or low in proportion to the percentage of sucrose, owing to the varying quantities of nonsugars peculiar to some beets. These points were strikingly brought out in the scatter diagrams. Table 2 gives the very significant value of $r_{1\ 30} = +0.81 \pm 0.01$, indicating that the percentage of sucrose increases with the refractometer reading. The regressions are linear, and the standard error of estimate ($S_y = 0.87$) is only 0.58, the value of the standard deviation. The total correlation coefficients describing this relationship between characters 1 and 30 for the separate lines were $+0.84 \pm 0.02$, $+0.86 \pm 0.02$, $+0.89 \pm 0.01$, $+0.86 \pm 0.02$, and $+0.85 \pm 0.02$. These results show in general that the percentage of sucrose increases directly with the refractometer reading. The correlation between characters 1 and 30 increases as the lines become more pure. If the purity of individual beets and the mean purity of lines are considered, this relationship becomes nearly perfect. The partial coefficients for this character admit of the same conclusions that were drawn for dry substance. (See character 16.)

CHARACTER 31.—The relative depth of the petiole groove represents the ratio of the depth to the width of the groove. The measurements were made from the petiole stamp. (See character 5.) The total correlation coefficients are not significant.

CHARACTER 32.—The relative flatness of the root was measured by dividing the fleshy surface (larger) diameter by the suture (smaller) diameter of the beet root. Character 32 is not significantly correlated with either character 1, 2, or 3.

CHARACTER 33.—The relative height of the foliage was represented by the proportion of the height of foliage to the diameter of foliage, measured in centimeters. The total correlation coefficients show that

the higher foliage types of beets have a slightly lower percentage of sucrose. (See character 19.)

CHARACTER 34.—Oblong leaves have been considered indicative of a high percentage of sucrose in the roots. The relative length of the leaf blade was defined as its length divided by its maximum width. The total correlation coefficients reveal no significant relations.

CHARACTER 35.—The relative length of leaf was expressed by the ratio of the blade plus petiole (length) to width of leaf. The coefficients in Table 2 for this character are not significant.

CHARACTER 36.—Vychinski (48) selected beets with long petioles, while Vivien (47) and Plot (34) favored the short robust petiole. Mass (17) considered this characteristic immaterial. The relative length of petiole was defined by the ratio of the length of petiole to length of leaf blade. Character 36 as here defined is not significantly correlated with either character 1, 2, or 3. Characters 33, 34, 35, and 36 were determined from measurements of the type leaf.

CHARACTER 37.—Certain investigators noted an increased percentage of sucrose in slender roots, while others found no significant relation. The relative length of root was expressed by the ratio of the length of the root to its maximum diameter. The weight of the beet has been found to increase with the length of the roots. (See character 24.) It will be noted that character 37 is positively correlated with character 1. (Compare with character 7.)

CHARACTER 38.—It is generally conceded that as the space between beets increases the percentage of sucrose decreases and the weight increases. Munerati, Mezzadrolì, and Zapparoli (22) and Pellet (31) found no relation between the available space and the weight of the beet. Many writers find that the beet yield per unit area increases with the space up to about 40 by 30 cm. and then decreases. This expected condition is due to the factor of the number of beets entering into the results. This cultural problem will not be considered in this investigation, which deals primarily with individual beets. The amount of space available for each plant during its vegetative period in the field was referred to as relative space. These measurements were made for each plant and were expressed in square centimeters of area. The total correlation coefficients of Table 2 and for the individual lines show that the weight and the sucrose content of the beet increases with the relative space but that the percentage of sucrose changes negligibly. The partial correlation coefficients confirm these results.

CHARACTER 39.—The percentage of sucrose in the beet increases with its ring density. The ring density was expressed by the ratio, number of vascular-bundle rings (see character 27) to the radius of the beet root measured in centimeters (after Seeliger (42)). Table 2 shows that this character is positively correlated with the percentage of sucrose, but negatively with weight and with the sucrose content. The partial coefficients confirm these results except for coefficient $r_{1\ 39\cdot2}$ which has the fault of holding a character constant that has partly been considered in the original data. (See character 22.)

CHARACTER 40.—Mehais (18) and Munerati (20) report that rooty beets have a higher percentage of sucrose, while Saillard (39) and Vivien (47) noted that they had lower percentages of sucrose and that rootiness was due to soil conditions. Other investigators (10) found no relation between the percentage of sucrose and the rooty

character. The beets were classified according to the number of large forked roots and not in relation to the finer roots of the suture groove. There were very few rooty beets in the material investigated and the coefficients are questionable.

CHARACTER 41.—Sugar beets having rough skins are reported to have a higher percentage of sucrose (47). The term "roughness of skin," describes the general character of the skin and not the surface irregularities which are due to underlying tissue growth. Character 41 is positively correlated with each of the characters 1, 2, and 3. (See character 9.)

CHARACTER 42.—Some investigators have noted that beets having curly leaves are higher in sucrose, while others have found the reverse relation. Fruwirth and others (10) state that neither character 1 nor 2 is correlated with character 42. Rugosity was the term used to describe the curliness of the beet leaf surface. As the rugosity of the leaf surface depends upon the age of the leaf and the external conditions, the type leaf was considered characteristic of the plant, and the precautions outlined under characters 12 and 18 were observed. The total correlation coefficients for the entire material studied are given in Table 2. The total correlation coefficients expressing the same relations for the individual lines were very different. For instance, line 3 gave correlation coefficients $r_{1\ 42}=0.0$, $r_{2\ 42}=0.0$, and $r_{3\ 42}=0.0$, showing that characters 1, 2, and 3, were absolutely independent of character 42. This was expected, because every beet in line 3 was uniformly alike for character 42.

CHARACTER 43.—The leaves of sugar beets vary as to the degree of neutral gray shade that appears along with their green color. (See character 9.) Table 2 indicates that beets having leaves of darker shade have higher percentages of sucrose. Character 43 is positively correlated with each of characters 1, 2, and 3. (See Tables 2 and 3 and character 9.)

CHARACTER 44.—Sugar marks are indicative of high percentages of sucrose. Sugar marks seem to be characteristic of sugar beets and are very rarely noticed on feed beets. These marks consist of corrugations on the beet surface that extend horizontally away from the root sutures. Character 44 is also positively correlated with each of the other characters 1, 2, and 3. (See Tables 2 and 3 and character 9.)

CHARACTER 45.—Mass (17) and Pritchard (35) noted that thick-leaved beets had higher percentages of sucrose than thin-leaved beets, whereas Bolotoff (2), Knauer and Doerstling (14), and Schribaux (41) noted the reverse. The thickness of the leaf was measured with a micrometer reading to 0.001 of an inch. The measurements were always taken from a particular locality on the type leaf because the thickness of sugar-beet leaves varies with the age of the leaf and changing external conditions. The partial coefficients indicate no significant relationships.

CHARACTER 46.—The majority of investigators have noted an increased percentage of sucrose in beets with spirally turned sutures. In these investigations no significant differences were noted as to characters 1, 2, or 3 between beets with straight and those with turned sutures.

CHARACTER 47.—It is generally acknowledged that the foliage weight per beet is negatively correlated with the percentage of sucrose but positively correlated with the weight of beet and the sucro

content. Schribaux (41) pointed out that the weight of leaves has increased with the breeding of beets having higher percentages of sucrose. The weight of the foliage represents only the quantity of foliage that was on each beet at the time of harvest and in no way represents the total weight of foliage produced by the beet during its vegetative period. Owing to the fact that the weight of the foliage on a beet depends upon its maturity, the weather conditions, and the losses of leaves during cultivation, as well as upon the total foliage produced, these results should be considered as to maturity of beet in connection with any physiological relation between characters. Thus the negative relation between characters 1 and 47 may either indicate that beets have low percentages of sucrose because they have retained most of their leaves and are not mature, or the reverse, that beets have high percentages of sucrose because they are mature and have lost most of their leaves. After due allowance is made for these two contingencies, there remains the possibility that beets having high percentages of sucrose may produce relatively less foliage.

CHARACTER 48.—Schneider (40) reported higher percentages of sucrose in beets having wide leaves, but Pritchard (35) reported the reverse relation. The width of the type leaf was considered characteristic of the plant. The total correlation coefficients of Table 2 show that the percentage of sucrose decreases as the width of the leaf increases. There is no doubt that the percentage of sucrose is negatively correlated with the width of leaf, because of the strong positive relation between characters 2 and 48 and the negative relation between characters 1 and 2. Further, if characters 1 and 48 are considered and the weight of the beets held constant, there appears to be no significant relation between the width of leaf and the percentage of sucrose ($r_{1\ 48\cdot 2} = -0.08 \pm 0.03$, Table 3.) This conclusion was significantly reached by a slightly different method. (See character 34 and Table 2.) The partial correlation coefficients should be considered in the light of the statements under character 15.

CHARACTER 49.—Pritchard (35) noted that beets having wide petiole grooves had smaller percentages of sucrose. The width of the petiole groove was measured at the point of minimum cross-section area of the petiole. (See character 5.) The total correlation coefficients are given in Table 2. The partial correlation coefficient $r_{1\ 49\cdot 2}$ supports Pritchard's conclusion.

CHARACTER 50.—Most breeders have associated the deep suture groove of sugar beets with high percentages of sucrose. The width of the suture was estimated by comparison with its depth. The coefficients for character 50 in Table 2 are not significant.

INTERPRETATION OF RESULTS

The multiple correlation coefficient of the sucrose content with the percentage of sucrose and the weight has the significant value of 0.965. This indicates that three-fourths of the variability has been considered, and the standard error ($S_{3\cdot 12} = 19.28$) is only about 25 per cent of the standard deviation for the sucrose content. As the sucrose content depends upon the percentage of sucrose and the weight of the beet root, the partial correlation coefficients for the percentage of sucrose with each of the selection characters listed in column 1 of Table 3 when constant for weight are given in column 2,

and the partial coefficients for weight with each of these characters when constant for percentage of sucrose are given in column 3 of the table.

Any permanent increase in the percentage of sucrose in the beet is of fundamental importance because a high percentage of sucrose is of value in itself, and the writer has shown under character 2, that the sucrose content increases with the percentage of sucrose ($r_{13:2} = +0.52 \pm 0.02$). The partial correlation coefficients $r_{1\ 11:2}$, $r_{1\ 13:2}$, $r_{1\ 17:2}$, $r_{1\ 24:2}$, $r_{1\ 28:2}$, $r_{1\ 41:2}$, and $r_{1\ 43:2}$ indicate that the percentage of sucrose of beets may be improved by selecting beets of increased density, dry substance, shoulder extension, hardness of tissue, length of root, purity, refractometer reading, roughness of skin, and darkness of leaf color. The negative coefficients $r_{1\ 21:2}$ and $r_{15:2}$ indicate that these beets should have flat crowns and petioles of small cross-section area.

In the consideration of lines of beets that are relatively uniform for percentage of sucrose, the partial correlation of the weight with the sucrose content constant for percentage of sucrose ($r_{23:1} = +0.96$) indicates a very significant relationship, because the selection of beets with characters significantly correlated with the weight will quite invariably be significantly correlated with the sucrose content. (Compare coefficients of columns 3 and 4, Table 3.) In this connection the selection characters 15, 18, 21, 24, 26, and 28, should be found of service. Some of these characters will present no difficulty because they are not significantly correlated with the percentage of sucrose. Others (character 18, for example) are positively correlated with the weight (+0.54) but negatively (-0.15) with the percentage of sucrose. This indicates that if the weight was increased by selecting beets of high foliage area the percentage of sucrose might be reduced. The alternative would be to increase the foliage area (character 18) by selecting beets with a large number of leaves (character 26) rather than beets with few large leaves (character 6).

In the study of lines that are uniform for either character 1 or 2, it is important to note the correlation of the sucrose content with each of the selection characters when either character 1 or 2 is held constant. The partial correlation coefficients for sucrose content with each of the selection characters constant for either percentage of sucrose (column 4) or weight (column 5) are given in Table 3.

The selection characters 9, 27, 41, 43, and 44 are positively correlated with the percentage of sucrose, the weight, and the sucrose content regardless of whether character 1 or 2 is held constant. The indications are that these particular characters should be further investigated to determine whether these relationships are or are not due to true genetic differences.

DISCUSSION

The correlations presented here are generally considered to be of a physiological nature and may be of value for indirect selection. Indirect selection permits of an easy determination of one character in place of the difficult determination of a valuable character if these two characters show a large significant correlation. Of the many selection characters studied, only a few were found sufficiently associated with the valuable characters of the beet to be of immediate

breeding value. As all selection characters were adapted to field conditions these few selection characters are of unusual consequence to the character and cost of sugar-beet breeding. The data presented show that the more important selection characters are of value for indirect selection and may be used to supplement the polariscope.

A review of the statistics on the percentage of sucrose and the weight of sugar beets shows that the coefficient of variation for weight is about five times greater than that for percentage of sucrose. These variations are largely of a modificational nature, being due to environmental conditions. Tables 2 and 3 show that weight is positively correlated with relative space (character 38) but percentage of sucrose is not significantly correlated with relative space. In the calculation of the partial correlation coefficients constant for weight, the larger part of these modifications is eliminated, and the consideration of these partial correlation coefficients as given in Table 3 should be utilizable for the selection and improvement of sugar beets.

As the weight and the sucrose content of the beet root increase with the space available per beet in the field, all beets planted for selection work should be spaced uniformly at the optimum distance. Indications are that this optimum distance varies with different strains of sugar beets. A uniform spacing of breeding beets at once obviates the necessity for discarding beets adjoining blank spaces in the row and the laborious determination and recording of the available space allotted each beet in the field.

As sugar beets tend to become uniform for one character or another and to fall into classes of high percentage of sucrose or of high weight, partial correlation may be applied directly to sugar-beet breeding. For example, sugar-beet strains that are of uniform weight or of high weight should be bred for an increased percentage of sucrose. The aim is to maintain the desirable weight characteristic of the strain and at the same time to increase the percentage of sucrose. Possibilities are offered by the method of partial correlation which provides a means of holding certain characters constant while other varying characters are considered. For instance, the partial correlations of the percentage of sucrose with each of the selection characters constant for weight (column 2, Table 3) indicate which selection characters should be used to improve the percentage of sucrose in this particular strain while its weight characteristic is retained. On the other hand, sugar-beet strains that have high percentages of sucrose and poor weight should be studied as to the partial correlation coefficients listed in column 3, Table 3. These partial correlation coefficients indicate which selection characters should be used for the improvement of sugar-beet strains that may be either of poor quality or weight. The choice of selection characters to be studied will depend upon the interrelation of the characters involved, as was illustrated on page 540.

As a strain becomes more uniform for a particular character, this character decreases in value as a correlation character for selection. When the strain is homogeneous for this character its correlation with other characters will show no correlation. (See character 42.) However, this particular character then becomes very useful as an identification mark of its strain.

SUMMARY

The material studied was statistically complete as noted. The combined material (496 individual beets) consisted of five previously known lines grown and examined under optimum conditions, measured in standard units, and analyzed immediately after harvest. All the data were recorded for each individual beet and the statistics calculated for the individual lines as well as the entire material. Scatter diagrams were made, and more than 500 total, multiple, and partial correlation coefficients were calculated, considering the percentage of sucrose, the weight, and the sucrose content of the beet with 47 selection characters.

A reliable and rapid method for estimating the number of sugar-beet leaves is given.

The selection character leaf density is defined as an exact method for stating the density of leaves on the sugar-beet crown.

The effects of the unequal spacing of beets in the field were eliminated by statistical methods.

In general the total correlation coefficients for each separate line are similar to the corresponding total correlation coefficient for the combined material.

Sucrose content was considered in relation to all of the selection characters because it is one of the most important characteristics of the sugar beet.

It appears that the physiological correlation of the percentage of sucrose with the weight of the beet is negative and significant. This conclusion is strengthened by the fact that nearly all the characters which are positively correlated with the percentage of sucrose are negatively correlated with the weight of the beet. Even though it was possible to show that the sucrose content increased with the percentage of sucrose when the weight of the beet is held constant, no partial correlation was calculated that showed a positive relation between the percentage of sucrose and the weight. In this connection it is obvious that if a line of beets is uniform for either weight or percentage of sucrose there would be no correlation between these characters. (See discussion, p. 541.)

Percentage of sucrose was found to be significantly and positively correlated with sucrose content.

As many of the correlations indicate a strong association of weight with sucrose content, the weight of the beet should be given more consideration.

These data showed that the percentage of sucrose is not significantly correlated with the available space given a beet in the field. As the weight and sucrose content of the beet increase with the available space, sugar beets planted for selection work should be spaced uniformly to the optimum distance for the strain.

As the multiple correlation coefficient and unpublished data indicate that the percentage of sucrose and the weight of beets are very important characteristics for determining the value of sugar beets, the partial correlation coefficients for each of these characters with each of the more important selection characters are given in Table 3. The partial correlation coefficients for sucrose content with these selection characters are also given.

The calculation of the partial correlations presented in this paper made it possible to state the net association of sugar-beet characteristics. These coefficients were used to analyze the selection characters which were highly correlated among themselves and to eliminate the effect of uncontrolled conditions. It also was found possible to apply partial correlation directly to sugar-beet breeding, because the percentage of sucrose and the weight of sugar beets seldom increase simultaneously.

The partial correlation coefficients of Table 3 also indicate which of the selection characters should be considered for the improvement of special strains of sugar beets that may be of poor weight or quality. They show likewise, that, aside from the polariscope, the refractometer reading (30) is the most reliable selection character to consider in improving the percentage of sucrose in the beet. Along with this, characters 17, 20, 24, 29, 41, and 43 should be considered.

The coefficients show that the number of leaves is the most important selection character influencing the weight of beets. Characters 13, 15, 18, 22, 24, 28, 39, and 48 might also be considered.

Characters 9, 27, 41, 43, and 44 were found to be positively correlated with each of the characters 1, 2, and 3.

The selection characters are adapted to field studies, and they can be utilized as a valuable aid in the improvement of sugar beets.

LITERATURE CITED

- (1) BLONSKI, F.
1893. AUSWAHL VON ZUCKERRÜBEN ZU ZUCHTZWECKEN NACH DEM VERHÄLTNISSE DER LÄNGE DER RÜBE ZU IHRER BREITE. Österr.-Ungar. Ztschr. Zuckerindus. u. Landw. 22: 927-939, illus.
- (2) BOLOTOFF, W.
1915. RECHERCHE SUR QUATRE LIGNES DE BETTERAVES. Zhur. Opytn. Agron. (Jour. Expt. Landw.) 16: 106-117. [In Russian. French résumé, p. 116-117.]
- (3) BRIEM, H.
1895. ZUR AUSWAHL DER RÜBEN IM HERBSTE MIT HILFE SELECTOREN. Österr.-Ungar. Ztschr. Zuckerindus. u. Landw. 24: 478-480.
- (4) ———
1903. BERICHT ÜBER FORTSCHRITTE UND NEUERUNGEN AUF DEM GEBIETE DES RÜBEN- UND RÜBENSAMENBAUES. Bl. Zuckerrübenbau 10: [33]-37.
- (5) COLIN, H., and GRANDSIRE, A.
1925. STRUCTURE ET CHIMISME DANS LA BETTERAVE. Compt. Rend. Acad. Sci. [Paris] 180: 599-601.
- (6) CORENWINDER, B., and CONTAMINE, G.
1878. DE L'INFLUENCE DES FEUILLES SUR LA PRODUCTION DU SUCRE DANS LES BETTERAVES. Compt. Rend. Acad. Sci. [Paris] 87: 221-222.
- (7) DOERSTLING, P.
1897. DIE RÜBENSAMENZUCHT. 46 p., illus. Berlin.
- (8) FLORIAN, A.
1923. EIGENSCHAFTEN UND ZUCKERGEHALT EINIGER RÜBENSORTEN. Ztschr. Zuckerindus. Czechoslovak. Repub. 47: 354-355.
- (9) FRIEDL, G.
1912. EIN BEITRAG ZUR FRAGE DER VERÄNDERUNG DER ZUCKERRÜBE WÄHREND DER AUFBEWAHRUNG. Österr.-Ungar. Ztschr. Zuckerindus. u. Landw. 41: 698-712.
- (10) FRUWIRTH, C., ROEMER, T., and TSCHERMAK, E.
1923. DIE ZÜCHTUNG DER VIER HAUPTGETREIDEARTEN UND DER ZUCKERRÜBE. In Fruwirth, C., Handbuch der Landwirtschaftlichen Pflanzenzüchtung, Aufl. 4, neubearb. Bd. 4, illus. Berlin.

- (11) GESCHWIND, L.
1900. SUR LES RELATIONS EXISTANT CHEZ LA BETTERAVE ENTRE LA GÉNÈSE DU SACCHAROSE ET LA STRUCTURE DE LA RACINE. *Rev. Gen. Chim. Pure et Appl.* 3: 465-476, illus. (Also published in *Bul. Assoc. Chim. Sucr. et Distill.* 18: 785-795. 1901.)
- (12) JANASZ, S.
1904. BESCHREIBUNG EINIGER ZUCKERRÜBENRASSEN. *Mitt. Landw. Inst. Breslau* 2: [913]-970, illus.
- (13) KAJANUS, B.
1912. GENETISCHE STUDIEN AN BETA. *Ztschr. Induktive Abstam. u. Vererbungslehre* 6: [137]-179, illus.
- (14) KNAUER, F., and DOERSTLING, P.
1894. UNTERSUCHUNGEN ÜBER RÜBEN MIT DUNKELGRÜNEM UND HELLGRÜNEM KRAUT. *Bl. Zuckerrübenbau* 1: 128-129.
- (15) KNEIFEL, R.
1895. FORMEN UND FORMENWECHSEL DES BLATTES DER ZUCKERRÜBE. *Osterr.-Ungar. Ztschr. Zuckerindus. u. Landw.* 24: [965]-973.
- (16) MAREK, G.
1886. UEBER DEN EINFLUSS DES BODENS AUF DIE ZUCKERRÜBENSAMENZUCHT. *Ztschr. Ver. Deut. Rübenzuckerindus. (n. F. 23)* 36: 51-101.
- (17) MASS, H.
1906. KORRELATIONSERSCHEINUNGEN BEI FUTTERRÜBEN. *Bl. Zuckerrübenbau* 13: 42-45, [49]-53, 67-70.
- (18) MEHAIS,
1868. ÉTUDE SUR LA BETTERAVE À SUCRE. *Compt. Rend. Acad. Sci. [Paris]* 66: 556-560.
- (19) MEYER, E.
1911. DIE ZÜCHTUNG AUF DER ERDE WACHSENDE ZUCKERRÜBEN. *Deut. Landw. Presse* 38: [279]-280.
- (20) MUNERATI, O.
1920. OSSERVAZIONI E RICERCHE SULLA BARBABIETOLA DA ZUCCHERO. *I. R. Acad. Lincei, Cl. Sci. Fis., Mat. e Nat. Mem.* 13: 175-322, illus.
- (21) ———
1923. CONTRIBUTION À L'ÉTUDE DE LA CONSTITUTION GÉNÉTIQUE DE LA BETTERAVE À SUCRE ACTUELLE. *Cong. Internat. Agr. [Paris]* 11 (2): 72-78.
- (22) ——— MEZZADROLI, G., and ZAPPAROLI, T. V.
1913. IL PESO E LA RICCHEZZA ZUCCHERINA DELLE BARBABIETOLE IN RAPPORTO ALLA SUPERFICIE A DISPOSIZIONE DELLE SINGOLE PIANTE NEL CAMPO. *Staz. Sper. Agr. Ital.* 46: 755-779, illus.
- (23) ——— MEZZADROLI, G., and ZAPPAROLI, T. V.
1915. L'INFLUENZA DELLA SFOGLIATURA SUL TENORE ZUCCHERINO IN BARBABIETOLE SINGOLARMENTE CONSIDERATE. *Staz. Sper. Agr. Ital.* 48: [743]-771, illus.
- (24) NOVOTNÝ, K.
1912. EIN BEITRAG ZU BETRACHTUNGEN ÜBER DIE BEZIEHUNGEN ZWISCHEN DEM PERZENTUELLEN ZUCKERGEHALTE UND DEM GEWICHTE DER RÜBEN. *Ztschr. Zuckerindus. Böhmen* 36: 269-272.
- (25) OETKEN, W.
1916. STUDIEN ÜBER DIE VARIATIONS- UND KORRELATIONSVERHÄLTNISSE VON GEWICHT UND ZUCKERGEHALT BEI BETA-RÜBEN, INBESONDERE BEI DER ZUCKERRÜBE. I. Teil. *Landw. Jahrb.* 49: 1-103. II. Teil. *Ztschr. Pflanzenzucht.* 3: [265]-333, illus.
- (26) PACK, D. A.
1923. TIME FOR TESTING MOTHER BEETS. *Jour. Agr. Research* 26: 125-150.
- (27) ———
1924. PERMANENT SPIRAL FOR TAGS. *Phytopathology* 14: 398-400, illus.
- (28) ———
1925. THE STORAGE OF MOTHER BEETS. CONDITIONS WHICH SHOULD BE OBSERVED IN STORAGE—SUGGESTED MODIFICATIONS IN PRESENT METHODS. *Facts about Sugar* 20: 874-875.
- (29) ———
1927. SPECIFIC GRAVITY SELECTION OF SUGAR BEETS. *Facts about Sugar* 22: 1281-1288, illus.

- (30) PEARSON, K.
[1924]. TABLES FOR STATISTICIANS AND BIOMETRICIANS. Ed. 2, Pt. 1, 143 p. [Cambridge, Eng.]
- (31) PELLET, H.
1914. SUR LE POIDS ET LA RICHESSE DE LA BETTERAVE PAR RAPPORT À LA SURFACE DE TERRAIN DONT ELLE DISPOSE. *Sucr. Indig. et Colon.* 84: 59-61, 84-88, 104-108, illus.
- (32) PITSCH, O.
1903. ERFABUNGEN UND RESULTATE BEI DER ZÜCHTUNG VON NEUEN PFLANZENRASSEN. *Deut. Landw. Presse.* 30: 415, 429-430, 440-441.
- (33) PLAHN, H.
1905. DAS ABBLATTEN DER RÜBEN. *Centbl. Zuckerindus.* 14: 134-135.
- (34) PLOT, J.
1897. DIE BETRACHTUNG DES RÜBENBLATTORGANS ALS VORARBEIT ZUR SELEKTION. *Bl. Zuckerrübenbau* 4: 314-319, illus.
- (35) PRITCHARD, F. J.
1916. CORRELATIONS BETWEEN MORPHOLOGICAL CHARACTERS AND THE SACCHARINE CONTENT OF SUGAR BEETS. *Amer. Jour. Bot.* 3: 361-376, illus.
- (36) PROSPOWETZ, E. VON, Jr.
1890. ZUR FRAGE DES INDIVIDUELLEN VERHALTENS DER ZUCKERRÜBE HINSICHTLICH DER ABNAHME DES ZUCKERGEHALTS. *Österr.-Ungar. Ztschr. Zuckerindus. u. Landw.* 19: 159-162.
- (37) REMY, T.
1914. FORMEN, SORTEN UND ZUCHTEN DER ZUCKERRÜBEN. *Fühling's Landw. Ztg.* 63: 752-769.
- (38) RÜMKER, K.
1894. EINIGES ÜBER ZUCKERRÜBENZÜCHTUNG. *Bl. Zuckerrübenbau* 1: 194-197, 217-219, 234-245, 262-265, 277-282, illus.
- (39) SAILLARD, E.
1912. LES BETTERAVES FOURCHUES ET RACINEUSES. *Jour. Agr. Prat.* (n. s. 23) 76: 149-150.
- (40) SCHNEIDER, J.
1895. ZUR CHARAKTERISTIK TYPISCHER ZUCKERRÜBENVARIETÄTEN. *Österr.-Ungar. Ztschr. Zuckerindus. u. Landw.* 24: 899-900.
- (41) SCHRIBAUX, E.
1915. PRODUCTION DES GRAINES DE BETTERAVES. LA SÉLECTION À LA FERME FEUILLE D'INFORMATIONS DU MINISTÈRE DE L'AGRICULTURE. *France Min. Agr., Off. Renseig. Feuille Inform.* Paris 20: 60, illus.
- (42) SEELIGER, [R. H.]
1920. ÜBER DIE RINGDICHTS ALS AUSLESEMERKMAL BEI DER ZUCKERRÜBE. *Mitt. Biol. Reihstatt. Landw. u. Forstw.* 18: 64-68, illus.
- (43) SENGBUSCH, R. VON
1926. VERGLEICHENDE UNTERSUCHUNGEN ÜBER WACHSTUMSRHYTHMUS, STICKSTOFFGEHALT U. ZUCKERLAGERUNG DER KLEINWANZLEBENER ZUCKERRÜBENZÜCHTUNGEN MARKEN ZZ, Z, N UND E. *Kühn-Arch.* 12: [104]-145, illus.
- (44) URBAN, J.
1915. ÜBER DIE SAFTREINHEIT EINZELNER ZUCKERRÜBEN. *Ztschr. Zuckerindus. Böhmen* 39: [151]-163, illus.
- (45) VELASCO, J. M. D. DE M. Y
1923. LA SÉLECTION ET L'AMÉLIORATION DE LA BETTERAVE SUCRIÈRE EN ESPAGNE. *Congr. Internatl. Agr. [Paris]* 11 (2): 78-86.
- (46) VILMORIN, J. L.
1923. L'HÉRÉDITÉ CHEZ LA BETTERAVE CULTIVÉE. 153 p., illus. Paris. (Thesis).
- (47) VIVIEN, A.
1920. SACCHAROGÉNIE, SÉLECTION DE LA BETTERAVE À SUCRE. *Bul. Assoc. Chim. Sucre et Distill.* 38: 143-163.
- (48) VYCHINSKI, J.
1894. DU RAPPORT EXISTANT ENTRE LA RICHESSE SACCHARINE DE LA BETTERAVE ET LES CARACTÈRES DES FEUILLES. *Bul. Assoc. Chim. Sucre et Distill.* 12: 383-389.

A MOSAIC OF WHEAT TRANSMISSIBLE TO ALL CEREAL SPECIES IN THE TRIBE HORDEAE¹

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INTRODUCTION

The green and the yellow types of mosaic discussed in this paper occur on winter wheat and winter rye in several counties in Illinois and Indiana (3, 4)² and on winter wheat in Davidson County, N. C. An extreme dwarfing or rosette condition is associated with the green mosaic on several varieties of winter wheat, and on this account the malady has previously been designated by the name rosette disease. Although this name characterizes the rosette condition, it appears that the more inclusive name mosaic is more appropriate.

The green and the yellow types of mottling associated with this malady may be caused by one virus or by distinct viruses. This point will be discussed later. Throughout this paper they are treated as a single disease on account of their close association in the infested soils that have been studied.

This mosaic shows certain characteristics which differ from those manifested by the tropical mosaic occurring on sugarcane, Indian corn, and other species belonging largely to the higher tribes of the grasses as classified by Hitchcock (1).

The most notable difference is in the matter of soil transmission. This mosaic of the small grains is perpetuated from year to year by means of virus present in the soil stratum (3, 4, 10, 11). As yet experiments have not supplied the evidence that might lead to the determination of the exact relationship between the soil and the virus. It is possible that subterranean vectors such as nematodes, soil-borne insects, other animal forms, or even fungi, may carry the virus. It is possible also that the virus is held by the finely divided particles of soil and organic matter.

The occurrence of this mosaic depends largely on the fall and winter conditions that influence growth and dormancy. The disease does not develop when susceptible varieties are sown in infested soil during the summer, and it is difficult to obtain infection experimentally from expressed juice when the inoculated plants are propagated in warm locations.

This mosaic has not been transmitted experimentally by *Aphis maidis* or by aphids which commonly feed on wheat. Its host range as determined thus far is within the tribe Hordeae, which is one of the lower tribes of grasses.

¹ Received for publication Sept. 24, 1929; issued March, 1930. These studies were conducted in part in cooperation with the Wisconsin Agricultural Experiment Station during the 7 years the writer was stationed there.

² Reference is made by number (italic) to "Literature cited," p. 556.

METHODS

Infested black-silt soil was obtained near Granite City, Ill., and infested brown clay-loam soil was obtained near Wanatah, Ind. All tests were made in these virus-infested soils, which were first transported to Madison, Wis., and later to the Arlington Experiment Farm, Rosslyn, Va. These soils have maintained a high degree of infestation. Some lots of the black-silt soils have been used each year for 10 years. In 1929 some of these showed signs of reduced infestation.

In previous experiments it was found that susceptible strains of Harvest Queen wheat and other varieties do not usually develop mosaic when sown in mosaic-infested soil in the spring when temperatures are rising; accordingly all sowings, including the spring types, were made in the fall at the period most suitable for sowing the fall grains.

In some instances each variety or species of host was tested in a row 4 feet long, and in others the rows were 2 feet in length.

All spring types were carefully mulched with straw or hay to prevent or reduce winterkilling. Although this mulching assisted greatly, some varieties were winterkilled entirely or in part.

Several Egyptian wheats and a barley were tested in 1929. These were obtained from the Egyptian Ministry of Agriculture through L. E. Melchers. Tests on these were of interest, as certain varieties found susceptible to rosette mosaic in the United States were resistant to a similar disease which occurs in the lower Nile region, thus indicating that the Egyptian disease may be due to another causal agent.

RESULTS

Tables 1 and 2 give results of all the susceptibility tests made with cereals other than winter common wheat and rye. The results of control tests made in virus-free soil are not tabulated. In all but one case the plants grown in the virus-free soil were free from mosaic and rosette. In one test on Red Winter spelt the control plot was close to virus-infested soil and 1 plant out of 46 developed yellow mosaic.

From Table 1 it is seen that all of the species and varieties of the different species of *Triticum* which were tested developed mosaic to some degree.

Three varieties of winter barley—Nakano Wase, Wisconsin Winter (C. I.³ 2167), and Han River—developed mosaic. The last two varieties showed a very small amount of the disease in 1926, but none developed in subsequent tests. Nakano Wase was the most susceptible barley tested. Persian 1, a spring barley, developed mosaic in 1929. No rosette occurred in barley.

All the varieties of winter oats tested were free of mosaic. They are: Bicknell (C. I. 206-155), Black (C. I. 691), Culberson (C. I. 273), Custis (C. I. 2041), Lee (C. I. 2042), Winter Turf (C. I. 431), Winter Turf (C. I. 453-4), Fulghum (C. I. 708), Fulghum (C. I. 699-2015), and Red Rustproof (C. I. 1079).

Both green and yellow types of mottling occurred on the small grains grown in virus-infested soil. The green type (fig. 1, B and C) was the most common. The yellow streaks, stripes, and blotches (fig. 1, D, E, G, H) were prevalent in Red Winter spelt and in certain selections of Currell, Illini Chief, Mediterranean, and Goens common wheats.

C. I. indicates a serial accession number of the Office of Cereal Crops and Diseases.

TABLE 1.—*Species and varieties of the genus Triticum, other than winter varieties of T. vulgare, which have been tested and found susceptible to a mosaic of winter wheat in experiments conducted at the Arlington Experiment Farm, Rosslyn, Va., in 1927, 1928, and 1929*

Species and variety	Growth habit	C. I. No. or source	Percentage of mosaic infection in—		
			1927	1928	1929
<i>T. vulgare</i> :					
Federation.....	Spring.....				75
Haynes Bluestem.....	do.....				6
Hard Federation.....	do.....				10
Hindi D.....	do.....	Egypt.			100
Marquis.....	do.....	3641	6	4	100
Sinal 1.....	do.....	Egypt.			5
Sonora.....	do.....	3036			100
<i>T. compactum</i> :					
Coppel.....	do.....	3068		4	10
Hybrid 60.....	do.....	5024		4	10
Hybrid 128.....	Winter.....	4512		75	80
<i>T. turgidum</i> :					
Titanic.....	do.....	5535		90	
Winter Alaska.....	do.....	5873		90	
<i>T. durum</i> :					
Aeone.....	Spring.....	5284			50
Beladi 26.....	do.....	Egypt.			100
Marouani.....	do.....				100
Mindum.....	do.....	5296			25
Palestine 12.....	do.....	Egypt.			100
Pentad.....	do.....				50
<i>T. dicoccum</i> :					
Black Winter.....	Winter.....	2337	2	12	1
<i>T. spelta</i> :					
Alstroum.....	do.....	1773		2	5
Bearded Winter.....	do.....	1724	8	6	60
Red Winter.....	do.....		100	98	100
<i>T. polonicum</i> :					
Abyssinian.....	Spring.....		3		100
Unnamed.....	do.....			60	95
<i>T. monococcum</i> :					
Unnamed.....	Winter.....		99	90	100

TABLE 2.—*Reaction of varieties of barley (Hordeum sativum) tested for susceptibility to a mosaic of winter wheat in experiments conducted at Madison, Wis., in 1926 and at the Arlington Experiment Farm, Rosslyn, Va., in 1927, 1928, and 1929*

Habit and variety	C. I. No. or source	Percentage of mosaic infection in—			
		1926	1927	1928	1929
Spring habit:					
Persian 1.....	Egypt.				40
Winter habit:					
Alaska.....	4106	0	0	0	
Han River.....	2163	4	0	0	0
Nakano Wase.....	754	4	4	16	95
Orel.....	351	0	0	0	
Pidor.....	901	0	0	0	0
Tennessee Beardless No. 6.....	2746	0	0	0	
Tennessee Winter.....	257	0	0		
Tennessee Winter Selection 66.....	3546	0	0		
Wisconsin Winter.....	2167	4	0	0	0
Do.....	2159		0	0	

The mottled areas of yellow mosaic consist of short or long, narrow or wide, patches which tend to parallel the veins of the leaf or the leaf sheath. Long streaks or stripes sometimes occur. In some cases these streaks consist of a series of short streaks that have become united. (Fig. 1, D, E, H.) In other cases continuous wide yellow bands appear between the veins of the leaf, leaving the veins and a narrow margin of the lamina tinged with light green, as shown in Figure 1, F.

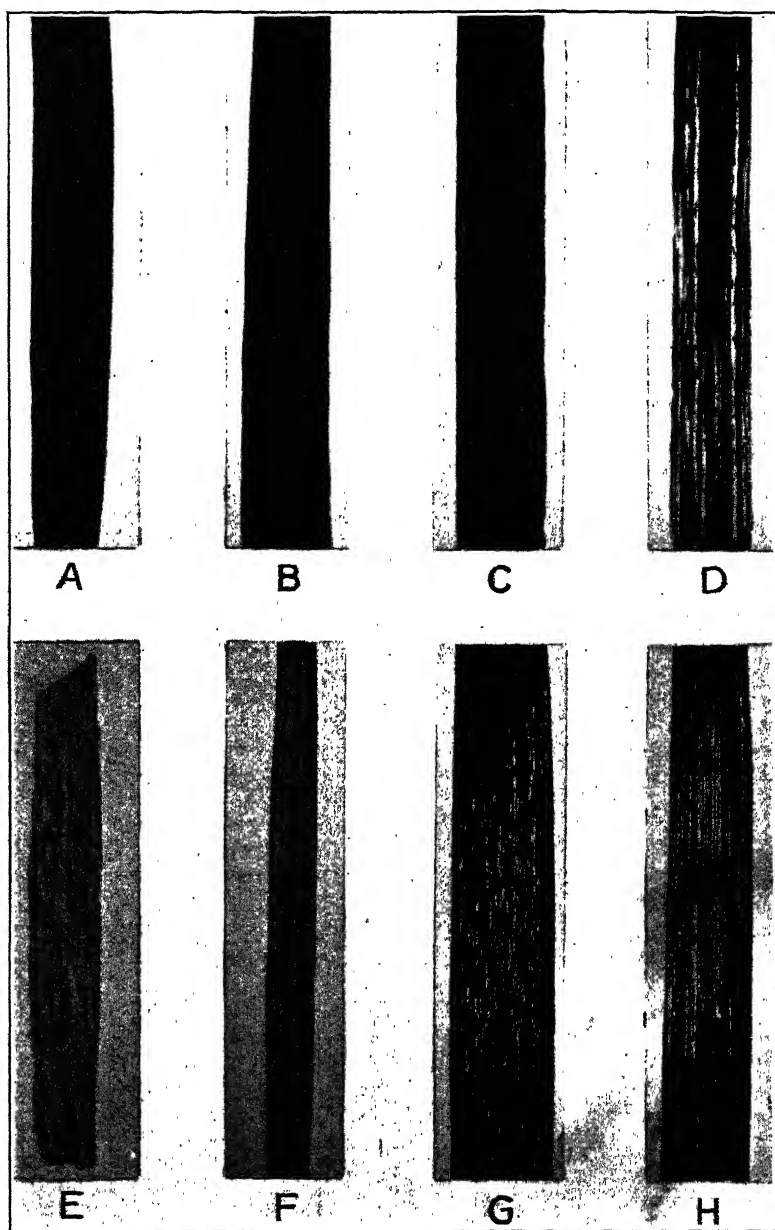


FIGURE 1.—Mosaic on small grains: A, Mosaic-free leaf of winter wheat; B, green mosaic on winter rye, showing a predominance of light-green surface; C, green mosaic on Harvest Queen winter wheat, showing a predominance of dark-green surface; D and E, yellow mosaic on Currell winter wheat; F, yellow mosaic on Polish wheat; G and H, yellow mosaic on a selection of Currell. A, C-E, $\times 1$; B, $\times 2$

The yellow patches, shown in Figure 1, D and E, frequently lose all of the carotin and xanthophyll pigments and become white or brown.

The yellow banding illustrated in Figure 1, F, has been observed only on Polish wheat. However, a less severe form (fig. 2, C) has been observed on individual plants of several varieties of common wheat grown in virus-infested soil.

The mosaic-free Polish wheat plants grown in the virus-free soil showed a considerable amount of light-green banding or striping on the leaves, as shown in Figure 2, B. This condition is more or less prevalent among certain varieties of mosaic-free common wheat, and individual plants have been observed which showed an unusual tendency to produce these bands under favorable environmental conditions. The extent to which this tendency may predispose plants to develop the yellow-stripe mosaic illustrated in Figure 1 is being studied.

Plants affected by severe yellow mosaic (fig. 1, D, E, and F) become stunted, but no excessive proliferation has been observed in these cases. In Currell and some strains of Illini Chief winter common wheat, and in Red Winter spelt, yellow mosaic frequently prevents the maturing of the seed. The grain from these plants is shriveled and of little or no value to the grower. In most cases the germination power of such seed is so low that it is necessary to conduct all selection studies in noninfested as well as in infested soil to insure obtaining seed for further experimentation.

In these experiments cell inclusions were abundant in the mottled leaves of all species and varieties of small grains affected by green mosaic, but they were usually very difficult to find or not present in tissues showing signs of yellow mosaic.

Modified or intermediate types of rosette have been observed in individual plants of rye and common wheat, as illustrated in Figure 3, B. These intermediate forms have also been observed in certain selections of Harvest Queen, Illini Chief, Mediterranean, and Goens common wheats. Affected plants become stunted at various times after typical rosette has made its appearance. The plants become dark green, thus masking most of the mosaic. If conditions are favorable such plants may send out secondary shoots, but they have been observed very rarely. These symptoms may be governed by factors that regulate infection and by genetic factors.

Selection studies show that many of the disease-free plants of the several species tested represent resistant types. Continual selection of yellow types and green types of mosaic shows that strains can be developed which produce a high percentage of plants susceptible to the mosaic type being selected.

DISCUSSION

Green and yellow types of mosaic have been observed on wheat outside of the infested areas given in this paper. In 1923 G. L. Peltier showed the writer a green mosaic on wheat growing in the experimental plots at Lincoln, Nebr., which was indistinguishable in general appearance from the green mosaic occurring in Illinois and Indiana. No rosette was associated with this mosaic, but this may have been due in part to the variety of wheat.

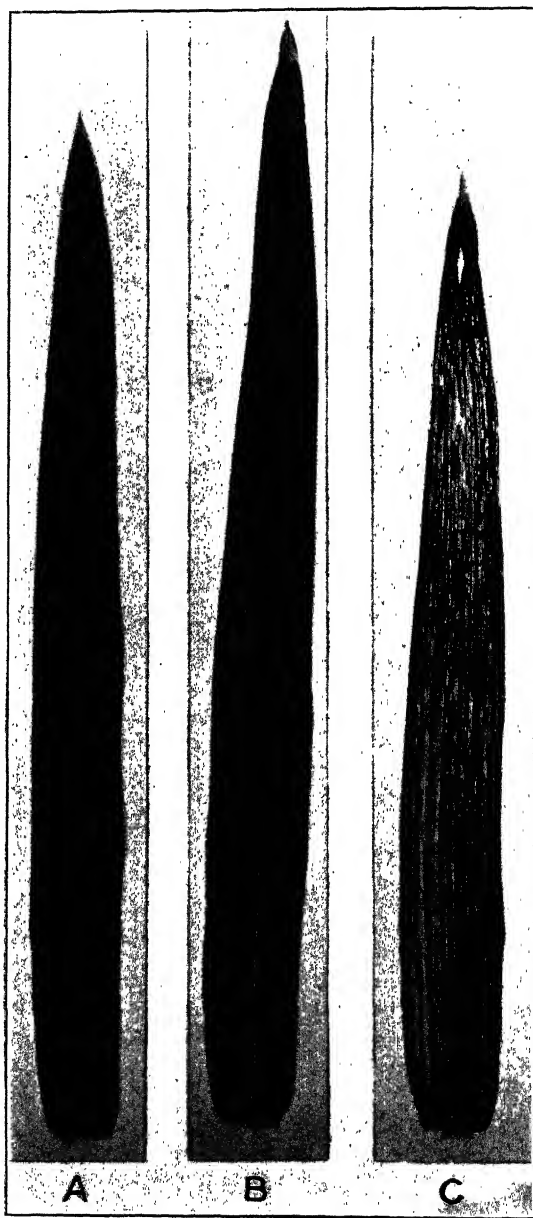


FIGURE 2.—A, Mosaic-free leaf of winter wheat; B, light-green banding associated with certain varieties and selections of *Triticum* when not infected with the virus of mosaic; C, yellow mosaic combined with the banding illustrated in B. All $\times 1$

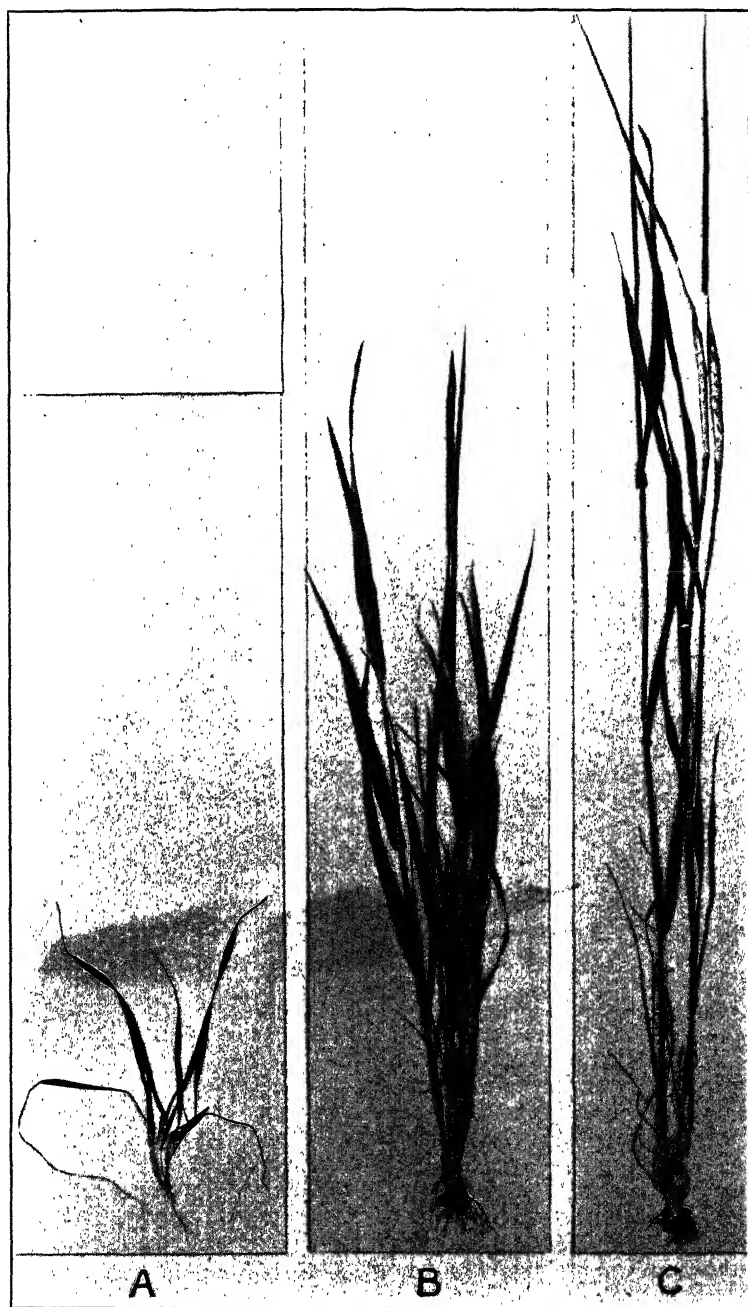


FIGURE 3.—Harvest Queen wheat. A, Typical rosette dwarfing, which appears early in the spring. The healthy control plants were three-fifths taller than the rosetted plants. B, Dwarfing and proliferation, which appear late in the spring or early in the summer. C, Healthy control plant collected with B. All $\times \frac{1}{4}$

Peltier reports that he transmitted this mosaic to wheat and to Indian corn by means of infectious juice and to one corn plant by means of an unidentified aphid.⁴ It seems evident, therefore, that the disease is infectious.

The writer made microscopic examinations of the mottled tissues of wheat plants collected at Lincoln, Nebr., but was unable to locate the typical cell inclusions which have always been found in similar tissues of plants with mosaic in Illinois and Indiana. These observations have led the writer to consider that the green mosaic in Nebraska is different from that occurring on wheat farther east.

In 1927 H. B. Humphrey collected a green mosaic on wheat near Rockville, Md. Mottled leaf tissues contained a large number of cell inclusions, but they were not exactly like those associated with the green mosaic which is the subject of the present investigation. The significance of this difference between cell inclusions must await further study.

In the spring of 1929 the writer's attention was called to a yellow mottling and stuntedness in winter wheat growing in the wheat propagation nursery of the Kansas Agricultural Experiment Station at Manhattan. Within a few days of this observation a similar mottling was observed in winter wheat on the agronomy farm of the Nebraska Agricultural Experiment Station located near Lincoln. No typical green mosaic was found associated with these yellow disorders.

The general appearance of these disorders was indistinguishable from the yellow mosaic that occurs in Illinois and Indiana. However, it is possible that these diseases are not due to a common cause.

Some of the patterns of yellow mosaic on wheat are strikingly similar in appearance to the streak and stripe diseases of Indian corn described and illustrated by Storey (9) and Stahl (8), respectively.

The mosaic situation in the small grains is suggestive of that occurring among several varieties of potatoes (7) and other species of Solonaceae (2, 5, 6).

Several forms of foliage mottling and dwarfing types of virus diseases which occur on the potato represent distinct diseases caused by distinct viruses. The work on the mosaics of tobacco, tomato, and *Nicotiana glauca* (2, 6) also shows clearly that there are several distinct viruses which produce different types of mosaic symptoms, and also that yellow mosaics are commonly associated with some of the green types (5, 6).

It is possible that several distinct viruses cause mosaic and other abnormalities in wheat, and that the cereal species, varieties, and selections vary as to their susceptibility to these different viruses. It is possible also that certain green and yellow types of wheat mosaic are commonly associated, as has been found to be the case with some of the green and yellow mosaics of tobacco (5, 6).

These are interesting analogies, but there are points concerning the grass mosaic under discussion which make it appear that they can not be pressed too far until methods of experimentation have been improved.

The interpretation of these varied expressions of mosaic, rosetting, and dwarfing depends to a large extent on the genetic purity of the

⁴HASKELL, R. J., and WOOD, J. I. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 27, p. 164-266, illus. 1923. [Mimeo graphed.]

host plants that produce the different types of symptoms. Long-continued selection for the botanical and agronomic characteristics of wheat varieties seems not to guarantee homozygosity for the factors determining resistance or susceptibility to mosaic and rosette. It has been found necessary to develop new lines pure for the disease characteristics that are under study. It also is essential that the small amount of natural crossing which occurs in wheat be eliminated by carefully bagging the heads of the plants.

Methods for separating and purifying the viruses of the grass mosaics must be developed.

SUMMARY

Infection experiments have been carried out with small grains sown in virus-infested soils transported from areas in Illinois and Indiana where wheat mosaic occurs.

The disease has been produced in the following species, all of which are of the tribe Hordeae: *Triticum vulgare*, *T. compactum*, *T. turgidum*, *T. durum*, *T. dicoccum*, *T. spelta*, *T. polonicum*, *T. monococcum*, *Hordeum sativum*, and *Secale cereale*.

All susceptible species tested gave evidence indicating that resistant strains were present.

Mosaic developed appreciably in cereals with the spring-growth habit only when they were sown in the fall at the time the fall types of cereals were sown. Careful mulching of the spring types reduced winterkilling.

Typical mosaic rosette occurred only among a limited number of varieties of winter common wheat. Strains of common wheat which develop 100 per cent typical mosaic rosette have been selected.

Green and also yellow types of mottling and yellow streaking or striping occur on plants growing in the infested soils studied.

By continual selection it has been possible to produce several lines of small grains which produce a very high percentage of yellow mosaic, and others which produce a high percentage of the green type.

The yellow types of mosaic cause the plants to become dwarfed late in the spring and the seed to be shriveled and practically valueless.

All varieties and species of small grains which produce mosaic when grown on the virus-infested soils under study were examined for cell inclusions. In all cases of green mosaic examined typical cell inclusions were abundant in the mottled leaves. Mottled leaves with yellow mosaic showed very few cell inclusions, and frequently none could be found.

A green mosaic with which no cell inclusions have been identified occurs in Nebraska.

A green mosaic occurs on wheat in Maryland with which cell inclusions occur, but these show characteristics somewhat different from those of the cell inclusions associated with the green mosaic occurring in Illinois and Indiana.

A yellow mottling occurs, apparently independent of green mosaic, on wheat at Manhattan, Kans., and at Lincoln, Nebr. The external signs of this malady are indistinguishable from those manifested by the yellow mosaic under study. No cell inclusions have been identified in the tissues of specimens obtained at Manhattan and Lincoln.

It is not possible to interpret all of these observations satisfactorily at this time. Several distinct viruses may cause mosaic and other disorders in the small grains. Some of the yellow and green mosaics of wheat may be commonly associated with each other, as has been found with some of the mosaics of the Solonaceae. Genetic factors within the plant may influence the symptoms produced by a given virus.

Rosette has never been observed on plants independently of mosaic, and all evidence indicates that rosette is a phase of the mosaic disease.

LITERATURE CITED

- (1) HITCHCOCK, A. S.
1920. THE GENERA OF GRASSES IN THE UNITED STATES WITH SPECIAL REFERENCE TO THE ECONOMIC SPECIES. U. S. Dept. Agr. Bul. 772, 307 p., illus.
- (2) JOHNSON, J.
1927. THE CLASSIFICATION OF PLANT VIRUSES. Wis. Agr. Expt. Sta. Research Bul. 76, 16 p., illus.
- (3) MCKINNEY, H. H.
1923. INVESTIGATIONS OF THE ROSETTE DISEASE OF WHEAT AND ITS CONTROL. Jour. Agr. Research 23: 771-800, illus.
- (4) ———
1925. A MOSAIC DISEASE OF WINTER WHEAT AND WINTER RYE. U. S. Dept. Agr. Bul. 1361, 11 p., illus.
- (5) ———
1926. VIRUS MIXTURES THAT MAY NOT BE DETECTED IN YOUNG TOBACCO PLANTS. Phytopathology 16: 893.
- (6) ———
1929. MOSAIC DISEASES IN THE CANARY ISLANDS, WEST AFRICA, AND GIBRALTAR. Jour. Agr. Research 39: 557-578, illus.
- (7) SCHULTZ, E. S., and FOLSOM, D.
1923. TRANSMISSION, VARIATION, AND CONTROL OF CERTAIN DEGENERATION DISEASES OF THE IRISH POTATO. Jour. Agr. Research (1923) 25: 43-118, illus.
- (8) STAHL, C. F.
1927. CORN STRIPE DISEASE IN CUBA NOT IDENTICAL WITH SUGAR CANE MOSAIC. Trop. Plant Research Found. Bul. 7, 12 p., illus.
- (9) STOREY, H. H.
1925. THE TRANSMISSION OF STREAK DISEASE OF MAIZE BY THE LEAF-HOPPER BALCLUTHA MBILA NAUDE. Ann. Appl. Biol. 12: 422-439, illus.
- (10) WEBB, R. W.
1927. SOIL FACTORS INFLUENCING THE DEVELOPMENT OF THE MOSAIC DISEASE IN WINTER WHEAT. Jour. Agr. Research 35: 587-614, illus.
- (11) ———
1928. FURTHER STUDIES ON THE SOIL RELATIONSHIPS OF THE MOSAIC DISEASE OF WINTER WHEAT. Jour. Agr. Research 36: 53-75.

REPETITIONAL DIPLANETISM IN THE GENUS PHYTOPHTHORA¹

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INTRODUCTION AND HISTORY

In a recent abstract (10)² concerning a prominently papillate species of *Phytophthora* different from *P. erythroseptica* Pethyb., as the cause of at least occasional instances of pink rot of potato (*Solanum tuberosum* L.) tubers in the United States, reference was made to the frequent manifestation of diplanetism by the fungus. Secondary motile zoospores were set forth as being delivered directly through an evacuation tube, or liberated from a 1-spored sporangium produced terminally on a delicate germ sporangiophore. Mention was made of the rather striking reproductive arrangement often resulting when zoospores imprisoned within an ordinary sporangium exhibit the latter type of development, as well as of the prevalence of diplanetism in congeneric forms, an appropriate instance being cited in another species which had been isolated from diseased potato tubers in the United States, and which was similarly capable of causing pink rot.

In spite of considerable study devoted to species of *Phytophthora* during more than a half century, the production of a second swimming spore from a previous one, without the interposition of a vegetative phase, appears, as far as the writer is aware, to have been recorded only once—in Sawada's recent account (22) of his *Phytophthora melongenae*. To be sure, according to Gäumaun (11, p. 80), Murphy observed

in *Phytophthora infestans* the shedding of the membrane, as in *Dictyuchus*; the zoospores which have found no suitable substrate come to rest and surround themselves with a membrane. After a certain time they again slip out with the same reniform appearance and swarm further.

From the publication (16, p. 459) cited, it is not apparent that the text permits such interpretation, as Murphy seemingly is not describing either any condition suggestive of *Dictyuchus* or emergence of a secondary swimming spore from an encysted stage. His account of the production of secondary conidia on germ tubes of zoospores is interesting nevertheless in relation to the phenomenon of repetitional diplanetism:

When the zoospores germinate under favorable conditions, the germ tube is of such enormously greater capacity than the original spore, and continues increasing in length (even though not completely filled with protoplasm), or at least remains alive and vigorous-looking so long, that a saprophytic existence is at once suggested. * * * They, too, have the faculty of producing "secondary" conidia, an observation which does not seem to have been recorded previously. * * * Subsequently in a preparation originally set up under sterile conditions and kept for five days at 10°-15° C., in which there had been abundant zoospore formation, the almost universal production of "secondary" conidia by zoospores situated near the edge of the cover glass was observed. * * * These new conidia

¹ Received for publication Nov. 13, 1929; issued March, 1930.

² Reference is made by number (italic) to "Literature cited," p. 572.

were borne on the ends of long spirally twisted tubes. They contained all the protoplasm, even of the branches, where such were present. In form they exactly resembled those produced by conidia being asymmetrical and provided with prominent papillae and yellow oil-drops, but they were much smaller, although having a considerably greater volume than the original zoospore.

The increased volume of the secondary conidium implies, apparently as an essential feature of the kind of development discussed, an expansion of material that could scarcely be attributed to purely reproductive processes.

A presumably similar course of development was noted later by Godfrey (12) in a study of one of the rhubarb (*Rheum rhaponticum* L.) foot-rot parasites described by him as *Phytophthora parasitica* var. *rhei*:

Within an hour or two after motility ceases zoospores often begin to germinate. * * * Germination in water sometimes results in the formation of small conidia, normal in appearance, as shown in F. These may produce zoospores, or may germinate by germ tubes and continue the vegetative growth.

The small conidium illustrated in the Figure F referred to is represented by a structure that would seem to exceed in volume the zoospore from which it originated by no less than eight times. The widening of the more distal portion of filament suggests too that the product of the zoospore may have attained such extension that it might perhaps have been more appropriately designated as a young mycelium rather than as a germ tube.

The reproductive development of the zoospores of *Phytophthora melongenae* described by Sawada does not appear to have been seriously disturbed through the intervention of vegetative growth. As the original account is in Japanese, a quotation from a somewhat free translation of the text³ may be appropriate:

After swimming about for 30 minutes or an hour the zoospore loses its cilia, comes to rest, and rounds up as a spherical body, 10 to 11 μ in diameter. On resting approximately 30 minutes it germinates by a single delicate germ tube, though in one instance two tubes were found produced. The germ tube either develops extensively, or it ceases to elongate and gives rise to a terminal conidium. The latter measures usually 11 to 12 by 8 to 9 μ , though examples measuring as much as 13 to 16 by 10 to 13 μ occur. Probably the former contains a single zoospore while the latter contains two. These small conidia open at the apex to liberate zoospores.

The zoospore which fails to make its way out of the conidium comes to rest inside and germinates, the germ tube sprouting through the papilla which already is open, or penetrating the conidial wall. In case the tube penetrates the wall, it frequently soon ceases elongation to produce a terminal conidium which germinates by liberating a zoospore.

Among the illustrations accompanying Sawada's text are three figures showing zoospores that have germinated by delicate germ sporangiophores each bearing terminally a minute, obviously 1-spored sporangium. In one of these figures (22, pl. 3, fig. 3) the structures are lying free; in another (22, pl. 3, fig. 6) the zoospores, four in number, imprisoned within the primary sporangial membrane, have each thrust their germ sporangiophore through the papillary opening to produce a minute sporangium externally; and in the third (22, pl. 3, fig. 7) four zoospores out of a larger number of imprisoned encysted structures have again each produced a minute zoosporangium internally, the germ sporangiophore here, however, in all instances perforating the wall of the primary sporangium.

³ The translation was prepared by Sabura Katsura.

To another figure (22, *pl. 3, fig. 4*) showing two evacuated zoospore membranes, each with an ample papillary opening, is attached the explanatory legend: "Resting zoospore producing a zoospore directly."

TREATMENT FAVORING DIPLANETIC DEVELOPMENT

As repetitional diplanetism seems to be of some importance in the biology of various species of *Phytophthora*, the present discussion supplementing Sawada's observations may not be excessive. The conditions favoring the production by encysted zoospores of a second swimming stage without the interposition of a vegetative phase apparently are the same as those that favor zoospore formation generally. In order that the necessary development might take place on a scale large enough to permit ready observation, it was found advantageous, after inducing the production and discharge of a generous supply of ordinary sporangia, to maintain conditions suitable for continued discharge. This could not usually be accomplished by mounting aerial sporangiferous material from artificial cultures on a microscope slide and covering with a cover glass, or by placing the material in a sealed hanging-drop preparation, or by cultivating in a Van Tieghem cell.

With the forms discussed in the present paper more satisfactory results were obtained through direct use of growth in Lima-bean agar, though for other types, like *Phytophthora cryptogea* Pethyb. and Laff., and *P. cinnamomi* Rands, this substratum was obviously less suitable. Portions of plate culture containing actively growing mycelium were removed to sterile Petri dishes, cut into pieces of convenient area, and irrigated with sterile distilled water in such a way that the upper surface of the agar was moistened without being inundated. As a result of such procedure, the papillate potato tuber parasite, for example, gave rise in the course of 12 to 15 hours to an extraordinarily heavy crop of sporangia, these being comparable in uniformity in size and shape with the sporangia produced in nature by *P. infestans* (Mont.) De Bary or *P. phaseoli* Thaxter. As long as the cover of the Petri dish was kept in place zoospore production occurred only in negligible quantity. On the removal of the cover the familiar changes recorded by numerous observers—cleavage of protoplasm, writhing movement, progressive individualization, swelling of the papillary substance—began and proceeded simultaneously in practically all the fully grown sporangia, with the result that after about a half hour very rapid liberation of zoospores lasting about 10 or 15 minutes ensued everywhere.

As an access of fresh air seemed to be almost the only consequence of the removal of the cover, Murphy's observation on the importance of oxygen in zoospore production in *Phytophthora infestans* was thus accorded confirmation in the behavior of the papillate pink-rot parasite. A similar response to ventilation was shown in preparations of most of the other congeneric organisms included in the present study. Material kept under observation and hence subjected to illumination from a microscope lamp gave rise to the swimming stage with noticeably greater rapidity than similar material in the same container but not exposed to special illumination.

SEQUENCE OF EVENTS IN DIPLANETIC DEVELOPMENT

With an abundance of zoospores available at the beginning, and additional quantities supplied by the dehiscence of succeeding crops of sporangia, the development leading to a secondary swimming stage could be looked for several hours later, suitable conditions being maintained meanwhile by admitting, if need be, fresh air from time to time, and by cautious replacement of water lost by evaporation. An early stage in such development of the papillate pink-rot organism is represented in Figure 1, A, *h* and *i*, the zoospores, after rounding up and becoming encysted as spherical bodies 8 to 12 μ in diameter, having produced a dome-shaped protrusion measuring mostly 3 to 4.5 μ in basal diameter and generally approximately the same in length. Until the protrusion attains its definitive size, it is filled, like the structure from which it originates, with protoplasm containing fine granules in rather open arrangement.

Since in considering the direct delivery of secondary motile zoospores the various forms included in the present study in which such delivery has been demonstrated exhibit no departure from a same uniform sequence of events, the series of stages (fig. 1, K, *l-n*) drawn from material of the rhubarb foot-rot fungus, *Phytophthora parasitica* var. *rhei* Godfrey, will illustrate the pertinent developmental stages revealed in the papillate pink-rot parasite as well as in other congeneric types. The first internal change readily noticeable consists in the retraction of the granular contents from the protuberance so that the latter appears to be filled only with clear liquid. (Fig. 1, E, *l*.) Soon thereafter, if not at the same time, the protoplast can be

EXPLANATORY LEGEND FOR FIGURE 1

A.—Unnamed form with papillate sporangia isolated from decaying potato tubers, possibly referable to *P. parasitica*: *a*, Extensive development of miniature sporangia from zoospores imprisoned within the second of a series of three sporangia; *b-e*, miniature sporangia produced from free encysted zoospores; *f*, an empty miniature sporangium; *h, i*, encysted zoospores with evacuation tubes; *j-s*, empty cyst envelopes after escape of secondary swimming spores.

B.—A nonpapillate species isolated from diseased Idaho potato tubers, possibly to be referred to *P. erythrospora*: *a-f*, Encysted zoospores, each showing a vacuole and an evacuation tube; *g*, motile zoospore at moment of liberation from cyst envelope; *h, i*, evacuated cyst envelopes.

C.—*P. melongenae*: *a*, Large sporangium containing an evacuated cyst envelope; *b*, sporangium containing imprisoned zoospores, of which all except one have given rise to germ sporangia; *c-j*, germ sporangia produced by free encysted zoospores; *k*, free germ sporangium after dehiscence; *l*, encysted zoospore with evacuation tube; *m, n*, evacuated cyst envelopes.

D.—*P. parasitica* derived from diseased cotton boll: *a*, Extensive development of miniature sporangia from imprisoned zoospores; *b*, sporangium containing two empty cyst membranes, one manifesting direct dehiscence, the other the production of a germ sporangium; *c*, sporangium with two evacuated cyst envelopes, and a secondary motile spore again to be retained within parent sporangium; *d*, sporangium with extensive development of germ sporangia from imprisoned zoospores; *e, f*, successive stages in development of germ sporangium; *g*, germ sporangium borne on an unusually long sporangiophore; *h, i*, germ sporangia after dehiscence; *j-n*, encysted zoospores with evacuation tubes; *o*, encysted zoospore immediately before liberation of secondary spore; *p*, escape of secondary zoospore from cyst envelope; *q-z, a', g'*, evacuated cyst envelopes.

E.—*P. parasitica* var. *rhei*: *a*, Sporangium containing two evacuated cyst envelopes; *b*, sporangium showing extensive production of miniature sporangia by imprisoned zoospores; *c*, sporangium with four imprisoned zoospores, three having produced germ sporangia, the other having discharged a secondary motile spore directly; *d-j*, germ sporangia after dehiscence; *k-n*, successive stages in development of secondary zoospore directly within cyst wall; *o*, zoospore with unusually long evacuation tube; *p*, another zoospore in same state as *l*, immediately preceding evacuation; *q-x*, evacuated cyst envelopes.

F.—Unnamed form isolated from diseased Honeydew melon: *a*, Germ sporangium produced by encysted zoospore; *b*, evacuated germ sporangium; *c-n*, evacuated cyst envelopes.

G.—Unnamed form isolated from diseased sugar-beet root in Utah: *a-c*, Encysted zoospores with broad protuberances of dehiscence; *d-m*, evacuated encysted envelopes.

H.—Unnamed form isolated from diseased tomato fruit in California: *a-e*, Encysted zoospores with developing evacuation tubes; *f-o*, evacuated cyst envelopes.

I.—*P. fagi*: *a*, Secondary motile spore after emergence being held fast by cilium adhering to inner surface of cyst wall; *b-e*, evacuated cyst envelopes.

J.—*P. citrophthora*: *a-c*, Germ sporangia produced by free encysted zoospores; *d, e*, evacuated germ sporangia; *f*, encysted zoospore with well-developed evacuation tube; *g, h*, evacuated cyst envelopes.

K.—*P. hibernatis*: *a-f*, Germ sporangia produced by encysted zoospores; *g-p*, evacuated germ sporangia.

L.—*P. cadourum*: *a*, Protrude development of germ sporangia by encysted zoospores imprisoned in a large sporangium; *b-d*, germ sporangia produced by free zoospores; *e*, germ sporangium after evacuation; *f*, encysted zoospore with evacuation tube; *g-k*, evacuated cyst envelopes.



FIGURE 1.—Repetitive diplanetism in various species of *Phytophthora*. All parts drawn with the aid of the camera lucida. $\times 500$
(For explanatory legend see opposite page)

observed to have drawn away slightly here and there from the cyst membrane. Writhing movements similar to those that are associated with sporogenesis in the large sporangia distinctive of the genus, now become apparent. Some time after movement becomes perceptible, usually not exceeding 10 or 15 minutes, the tip of the protrusion gives way and the protoplasmic contents pass out through the isthmus (fig. 1, E, *m*), becoming integrated at the orifice as a biciliate zoospore of the same type as that from which it was derived (fig. 1, E, *n*). The passage of the protoplast through the relatively wide neck is ordinarily accomplished in 3 or 4 seconds, while the pause between its delivery and its swimming away as a motile zoospore occupies about 1 second as a rule, rarely more than 2 seconds.

As the zoospore produced is not apparently distinguishable from those coming directly from an ordinary large sporangium, only the empty cyst membrane with its papillary orifice or open evacuation tube—a structure much more familiar to students of the Saprolegniaceae than to investigators dealing with the general run of fungi pathogenic to economic plants—is left behind as visible evidence of the developmental phenomenon described, which for convenience might be referred to as “direct” diplanetism. The evacuation tube, of course, represents the membrane surrounding the cylindrical portion of the protuberance of dehiscence, and is generally relatively short, often measuring about 1μ in length. (Fig. 1, A, *n*, *g*, *r*.) Greater lengths, however, are not rare (fig. 1, A, *k*, *l*, *m*, *o*, *p*, *s*), although a condition like that shown at A, *j*, the most extreme encountered in any of the forms studied, in which the length of the tube equals or perhaps slightly exceeds the diameter of the spherical part, must be regarded as decidedly unusual. Obviously the empty cyst envelopes remaining as evidence of the occurrence of direct diplanetism correspond to the two structures shown in Sawada's Plate 3, Figure 4, to which reference has already been made.

The other kind of repetitional diplanetetic development illustrated by the Japanese author and described in his text somewhat more fully, was, as has been stated earlier, also abundantly represented in material of the papillate pink-rot fungus. The encysted primary zoospore served as origin of a filament from 1 to 1.3μ in diameter growing out either immediately from its periphery (fig. 1, A, *c*) or more frequently from the tip of a much stouter protuberance (fig. 1, A, *b*, *d*, *e*) similar to the modification involved in the direct dehiscence already described. After attaining a length ordinarily varying between 5 and 25μ , though sometimes exceeding 50μ , the delicate filament ceased to elongate and swelled terminally into an expansion which increased in size with the migration into it of granular materials. Eventually the protoplasmic contents were completely transferred to the terminal structure, which then invariably became delimited from the filament by a septum. The distinctive shape, often obpyriform, and the terminal papillate modification that soon became apparent at once characterized the structure externally as a miniature sporangium. This characterization was consistently borne out when, after perceptible retraction of the contents from the confining membrane, especially in the apical region, and writhing movements of the usual duration, the apical beak yielded, and the protoplast squeezed through the orifice and swam away from the empty sporangium (fig. 1, A, *f*) as a biciliate zoospore. The latter was not distinguishable from

zoospores of primary origin, although in pronounced instances it seemed to be marked by perceptible inferiority in size. The developmental feature incident to the production of a secondary motile zoospore formed singly within a miniature sporangium borne on a delicate germ sporangiophore arising directly from an encysted zoospore or from an ineffective protuberance of dehiscence may conveniently be termed "indirect" diplanetism.

As has been suggested, indirect diplanetism is often revealed conspicuously in the case of zoospores that have failed to escape from the large primary sporangia usual in species of *Phytophthora*. Incomplete discharge of the latter structures, to which reference is made by numerous investigators, is observable in some measure in almost every lot of material of any member of the genus. While the reasons for frustrated dehiscence are not always obvious, in the writer's experience instances became especially numerous when the upper surface of the irrigated Lima-bean agar over which the sporangia were distributed either became too dry or was flooded to excess. It seems probable, therefore, that deficiency in moisture on the one hand and deficiency in oxygen on the other are frequently causes. In any case the imprisoned zoospores, after moving about within the sporangium for a considerable period with such vigor as their number and the limited space permitted, finally came to rest and rounded up inside.

According to observations of numerous students, further development proceeds by the production of vegetative germ tubes that perforate the sporangial wall and thus gain access to the exterior. Such vegetative development is common, especially when the structures concerned remain submerged rather deeply. Under more favorable circumstances, as has been mentioned, imprisoned zoospores of the papillate pink-rot fungus were found to give rise individually to single germ sporangiophores that perforated the sporangial wall, ordinarily without evident diminution in diameter, or passed through the papillary orifice. A terminal miniature sporangium that subsequently liberated a single secondary swimming spore was produced in each case. In instances where the imprisoned zoospores numbered a score or more, or where only a relatively small proportion had been discharged normally, the encysted bodies were packed so closely that their membranes partly adhered to one another, and the germ sporangiophores were thrust forth in bristling array. (Fig. 1, A, a.) The resulting arrangement provided a reproductive apparatus of distinctive appearance.

Direct diplanetism, while usually not as abundantly manifested in the case of imprisoned zoospores of the papillate pink-rot fungus as indirect, nevertheless is observed here in considerable measure. As the circumstances attending such occurrence are evidently similar in all species where it has been demonstrated, figures drawn from congeneric forms may be taken as adequately illustrative. When only one (fig. 1, C, a) or a few (fig. 1, E, a) zoospores are imprisoned, so that the cysts come to lie within the sporangial envelope under conditions not greatly different, with respect to crowding, from those encountered outside, all of the secondary swimming bodies may be directly discharged by way of an evacuation tube and gain access to the exterior of the sporangial wall by passing through the papillary opening. Direct repetitional development may occur even in cases where the papillary opening happens to be effectually blocked by one

of the cysts, as for example, in the instance represented in Figure 1, D, C. The obstructing cyst readily discharged its protoplast by the happily oriented evacuation tube directly through the papillary orifice, while the secondary zoospore from the less fortunately placed cyst was prevented from egress and as a result after protracted effort rounded up inside. In most instances, perhaps, the majority of the zoospores imprisoned within a sporangium give rise to a secondary swimming stage by the indirect method of development, while one or a few, especially of those most favorably situated with reference to crowding and accessibility to the papillary opening, manifest direct dehiscence. (Fig. 1, D, *b*, and E, *c*.)

PARTICULARS OF DIPLANETISM IN VARIOUS SPECIES OF PHYTOPHTHORA

Diplanetism is not restricted to a few members of the genus *Phytophthora*. Its occurrence in a fungus isolated from diseased potato tubers originating in Idaho in 1922 was recorded earlier. Mainly because of the identity of the host material from which it was derived, its ready pathogenicity to potato tubers on inoculation into wounds, and the absence of any papillate modification in the ovoid sporangia, the fungus was tentatively referred to *P. erythroseptica*. The American nonpapillate pink-rot fungus was never found to produce sexual structures in any substratum on which it was tried out, though parallel cultures of the organism received from the Centraalbureau voor Schimmelcultures at Baarn, Netherlands, under the label "*P. erythroseptica*" (presumably authentic, inasmuch as it was contributed originally by G. H. Pethybridge), often yielded an abundance of normal oospores. The sporangia of the American form exceeded those of the European parasite, especially in length, measurements of 200 of these bodies developed on irrigated Lima-bean agar giving an average length of 53.5μ and an average diameter of 30.2μ as compared with 44.5μ and 29.7μ , respectively, calculated for the organism obtained from abroad. A rather strong proliferous tendency manifested in the American fungus by the sporangiophore growing through the base of one sporangium after its evacuation, to produce another farther on, often yielded a series of three or four empty sporangial envelopes borne on the same axial sporangiophore. The zoospores of the fungus are relatively large, measuring in the subspherical encysted form about 12μ in diameter. Development leading to a second swimming stage (fig. 1, B, *g*) is begun by the production of a papilla of dehiscence usually 4 to 6.5μ in diameter and 2 to 8μ in length (fig. 1, B, *a-f*), the empty envelope being provided with a rather wide evacuation tube of variable length (fig. 1, B, *h-o*). Indirect diplanetism through the development of a miniature 1-spored germ sporangium has never been observed in the fungus under consideration.

Irrigated pieces of Lima-bean agar culture of the parasite isolated by Ocfemia (17) from diseased eggplant (*Solanum melongena* L.) fruits in the Philippines, and treated by him as being identical with *Phytophthora melongenae*, provided an abundant display of diplanetism. (Fig. 1, C, *a-n*.) Production of miniature sporangia (fig. 1, C, *c-j*) appeared much more frequently than direct liberation of the secondary motile bodies, both when the encysted primary zoospores were free and when they were imprisoned within the large sporangia.

(Fig. 1, C, *a*, *b*.) The secondary miniature sporangia produced by two of the seven imprisoned zoospores shown in Figure 1, C, *b*, did not give rise to a swimming stage directly, but gave rise instead to tertiary sporangia. Additional irregularities were found in occasional branching of the germ sporangiophore, the short diverticulum not set off by a cross wall and the relatively long element eventually delimited by a septum, shown in Figure 1, C, *c* and *i*, respectively, providing representative examples.

Abundant diplanetism was evident in irrigated material of a fungus communicated by S. F. Ashby as a strain of *Phytophthora parasitica* Dastur derived from cotton bolls in Monserrat. It, presumably, was the same organism further referred to in a publication (1) as having been isolated by E. M. Wakefield. (Fig. 1, D, *a-e'*.) Many of the larger sporangia of the fungus, suggesting chlamydospores in their almost subspherical shape, are provided with a relatively thick envelope. Apparently because of its substantial character, the latter structure is not readily perforated by the germ sporangiophores of imprisoned zoospores, which consequently are constrained to wind about extensively before emerging either by eventually piercing the wall or more often by passing through the papillary opening. (Fig. 1, D, *a*, *d*.) Failure to reach the exterior is rare, since the germ sporangiophores, even when not confined, often attain an unusual length. (Fig. 1, D, *g*.) The broad basal protuberance from which many (fig. 1, D, *e*, *f*, *g*, *i*), though not all (fig. 1, D, *h*), of the germ sporangiophores arise give an impression as if direct development of a second swimming stage is first attempted, followed in case of failure by the more indirect development. That the acquisition of cilia within the membrane of the encysted structure (fig. 1, D, *o*) and the escape of the biciliate zoospore (fig. 1, D, *p*) are not rare is attested by the presence of numerous empty cyst envelopes with evacuation tubes of variable length, abundantly scattered about free in appropriately treated material, as well as within empty primary sporangia (fig. 1, D, *b*, *c*).

Close similarity to the cotton-boll fungus in structures associated with diplanetism as well as in cultural characteristics and morphology of the sporangium was revealed in eight cultures that had been isolated from the tomato, five having been obtained from fruits affected with buckeye rot, two from stems affected with decay near the soil line, and the other from a rootlet showing decay at the tip. Such similarity is in harmony with the assignment of the buckeye-rot parasite, originally described from the tomato as *Phytophthora terrestris* Sherb., to *P. parasitica*. About the same similarity was evident also in a fungus derived from rhubarb affected with foot rot in the District of Columbia in September, 1928, and presumably to be identified with the form described by Godfrey as *P. parasitica* var. *rhei*. Here, also, frustrated dehiscence of the zoospores was provided for through the appearance of a secondary swimming stage, by direct discharge of the motile body (fig. 1, E, *a*), by production of a germ sporangium (fig. 1, E, *b*), or by both types of development (fig. 1, E, *c*). The sporangial wall usually was not as thick as the more strongly indurated homologous structures of the cotton-boll parasite, its perforation by the germ sporangiophores entailing only relatively little coiling of the filamentous structures. Otherwise production of the germ sporangia (fig. 1, E, *d-j*) took place much as in the cotton-boll

fungus, and the direct liberation of zoospores from the encysted bodies (fig. 1, E, *k-n*, *p*), following a sequence of stages already described, yielded evacuated cyst envelopes in large numbers (fig. 1, E, *q-x*).

A moderate display of diplanetism was observed in irrigated Lima-bean agar preparations of the species of *Phytophthora*, the isolation of which from a honeydew melon (*Cucumis melo* var. *inodorus* Naud.) fruit affected with decay was reported in a brief abstract (9). Production of germ sporangia (fig. 1, F, *a*, *b*) occurred less frequently than liberation of the secondary motile spores from the primary encysted ones (fig. 1, F, *c-n*). When the latter were somewhat irregular in shape, often as a result of rounding up under crowded conditions in insufficient water, their capacity to give rise to a second swimming stage was not noticeably impaired. (Fig. 1, F, *d, f*.)

A species of *Phytophthora* isolated by C. M. Tompkins from a mature sugar beet (*Beta vulgaris* L.) root originating at Price, Utah, in 1927, and greatly resembling the form isolated from affected Idaho potato tubers already discussed with respect to cultural characters and to morphological features pertaining to the sporangium, resembled the latter fungus also with respect to repetition of the swimming stage. The relatively large encysted primary zoospore, measuring usually about 12μ in diameter, put forth a wide papillary protuberance (fig. 1, G, *a-c*) that functioned in permitting the direct liberation of a secondary motile spore, leaving the empty cyst envelope (fig. 1, G, *d-m*) provided with a usually short evacuation tube. Germ sporangia arising from encysted zoospores were never observed. The funnel-shaped evacuation tube shown in G, *j*, and the short cylindrical diverticulum with the distal part delimited by a septum shown in G, *k*, represent departures from more regular development. In the absence of a more adequate description of the beet parasite, measurements of 50 sporangia produced on irrigated Lima-bean agar gave a range in length of 34 to 75μ , with an average of 58.3μ , and a range in width of 27 to 40μ , with an average of 33.1μ . After the evacuation of the somewhat narrow ovoid zoosporangium by means of an apical modification not protruding from the general contour as a recognizable papilla, the sporangiophore often continued growth through the empty envelope to produce another sporangium farther on. Repetition of such renewal of growth frequently resulted in three or four empty envelopes being found in series on a single axial supporting filament.

A species of *Phytophthora* isolated by G. B. Ramsey in October, 1928, from California tomato (*Lycopersicum esculentum* Mill.) fruits with brownish or brownish-purple lesions on the distal region showed great similarity in its manifestation of diplanetism to the congeneric fungi from Utah sugar beets and Idaho potatoes. The primary zoospore, measuring usually from 10 to 12μ in diameter after encystment, produced a protuberance of ample size (fig. 1, H, *a-e*) by means of which the motile secondary spore was directly liberated, leaving behind the empty cyst envelope with open evacuation tube (fig. 1, H, *f-o*). The fungus with its ovoid nonpapillate sporangia having an average length of 47.3μ and an average diameter of 30.9μ —these values being calculated from 50 measurements, using irrigated Lima-bean agar—was apparently specifically distinct from any hitherto reported on the tomato. The absence of protruding papillae from the sporangia readily sets it apart from the types assignable to

Phytophthora parasitica (*P. terrestris* and the stem-girdling fungus reported by Reddick (20)), as well as from *P. infestans* (Mont.) De Bary and *P. mexicana* Hotson and Hartge (13). Compared with an authentic culture of *P. cryptogea* Pethyb. and Laff. received from the Imperial Bureau of Mycology, the California fungus showed a considerably faster rate of growth, a noticeably smaller diameter of mycelial threads, and a vastly greater production of sporangia. Its close affinity and possible identity with the parasites from sugar beets and from Idaho potatoes were indicated by similarity in behavior and appearance when the three organisms were given similar treatment in parallel cultures and preparations.

Repetitional diplanetism in moderate measure was revealed in irrigated Lima-bean agar preparations of a fungus received in artificial culture from the Centraalbureau voor Schimmelcultures as *Phytophthora fagi* Hartig. The secondary zoospore in all cases observed was formed directly within the cyst envelope. In a number of instances the protoplast, after its passage through the papilla of dehiscence, was held tethered near the orifice for several minutes by one of the cilia adhering to the inner surface of the empty cyst membrane apparently with sufficient firmness to resist the pull exerted by the other cilium. (Fig. 1, I, a.) Since *P. fagi* has frequently been regarded as probably identical with *P. cactorum* (Cohn and Leb.) Schroet., and indeed resembles it closely in shape and size of sporangium, it may be of interest to note that in the fungus under consideration a secondary swimming stage was not observed to arise indirectly through the production of a miniature germ sporangium—a type of development exceedingly frequent in *P. cactorum*. The encysted zoospores of the fungus from the Centraalbureau and the empty envelopes left behind after their evacuation (fig. 1, I, b-f) appeared generally slightly larger than the corresponding structures of the related organism, the average diameter being about 10μ in the former, as compared with 9μ in the latter. Differences in cultural features were also evident, the fungus received from the Netherlands growing less than one-fourth as rapidly in linear extension as the other when cultivated, for example, on Lima-bean agar, and its thallus revealing a densely branching habit on that medium as compared with an openly disposed one. Any trustworthy opinion regarding the relationship of *P. fagi* and *P. cactorum* should, however, be based on a study of a dozen or more cultures of the beech (*Fagus sylvatica* L.) seedling parasite, preferably from sources not too close together geographically.

Several strains of *Phytophthora citrophthora* (Sm. and Sm.) Leon., all derived presumably from California lemon (*Citrus limonia* Osbeck) fruits affected with brown rot, exhibited repetitional diplanetism in ample measure in irrigated Lima-bean agar preparations. The secondary motile stage was developed both indirectly by the production of germ sporangia (fig. 1, J, a-e) and directly by the acquisition of cilia within the cyst membrane. The evacuation tube associated with the latter process was usually of moderate proportions. (Fig. 1, J, f-h.) As in certain other species, the germ sporangiophore frequently was present as a delicate prolongation of a broader basal protuberance, the latter evidently being an unsuccessful evacuation tube. (Fig. 1, J, a, c-e.)

Diplanetism was abundantly manifested in irrigated Lima-bean agar preparations of a fungus received from the American Type Culture

Collection as *Phytophthora hibernalis* Carne, and representing, presumably, an authentic type of the organism described recently (3) as the cause of brown rot of citrus in Australia. The secondary swimming stage was brought about through the production in extraordinary numbers of miniature germ sporangia (fig. 1, K, *a-f*) and their subsequent evacuation (fig. 1, K, *g-p*). No instances of development of motility directly within the cyst envelope were observed, though the frequent origin of the delicate germ sporangiophores from a broader basal protuberance (fig. 1, K, *a-c, f, m, n, p*) suggested the possibility that such development may have been attempted but came to naught, owing perhaps to unsuitable conditions. The production of a tertiary germ sporangium from the secondary one shown in Figure 1, K, *a*, and a certain tendency toward branching evident in some sporangiophores (fig. 1, K, *a, d, g, h, l, o*) constitute irregularities.

Reference has been made to the frequent exhibition of diplanetism in irrigated preparations of *Phytophthora cactorum* through the production of miniature sporangia. The important part played by such development in the liberation of swimming zoospores in instances of frustrated dehiscence of the large sporangia is illustrated in Figure 1, L, *a*, showing 28 germ sporangia borne on pedicels thrust through the confining sporangial wall from a corresponding number of imprisoned cysts. Similar development ensues in quantity also with primary zoospores that have encysted unconfined in their surroundings after a normal period of active swimming. (Fig. 1, L, *b-e*.) The secondary zoosporic stage may arise, too, through the fashioning of the motile body within the cyst membrane, as shown by the presence of a papillate protuberance in many encysted structures (fig. 1, L, *f*), later persisting on the evacuated envelope as a cylindrical modification of varying length (fig. 1, L, *g-k*). While all strains of *P. cactorum* show close similarity in diplanetic development, it may not be amiss to record that the illustration shown in Figure 1, L, was made from a Lima-bean agar preparation of one of the several strains from diseased stems of *Lilium pyrenaicum* Gouan, the isolation of which was reported earlier in a brief note (7).

COMPARISON WITH DIPLANETIC DEVELOPMENT IN RELATED FUNGI

Although diplanetism has been observed in additional representatives of *Phytophthora*, no features associated with the phenomenon have come to light which are not amply illustrated in the dozen forms mentioned. If the papillate pink-rot fungus be assigned to *P. parasitica*, which, in spite of certain differences, it would seem to resemble more closely than any other of the better established species, and the fungi from Idaho potato tubers, Utah sugar-beet roots, and California tomato fruits be regarded as conspecific, the assortment of forms would be resolved presumably into about eight species. In all of the forms considered, the fashioning of the secondary zoospore took place directly within the cyst envelope or within the miniature sporangium, accompanied by noticeable writhing movements with retraction of protoplasm here and there from the confining wall, especially at the papilla or evacuation tube. The biciliate body after its passage through the open protuberance immediately swam away, except, as has been mentioned, in a few instances where a cilium adhered to the inner surface of the cyst wall. The manner of dis-

charge was therefore essentially the same as that generally characteristic of the ordinary sporangia produced throughout the genus.

The evident parallelism between the primary and the secondary swimming stages with respect to the fashioning and discharge of the motile zoospores in species of *Phytophthora* suggests analogous development in the genus *Pythium*. In discussing the germination of the zoospores of his *Pythium dictyosporum*, a species with filamentous sporangia parasitic on *Spirogyra insignis* Hass, Raciborski (19, p. 284) stated that if the germ tubes do not reach the *Spirogyra* filament, a new, small sporangium, which contains only one zoospore, is formed at the tip of the germ tube.

The small sporangia to which reference is made correspond apparently to the miniature germ sporangia produced by most of the *Phytophthora* forms discussed here. No details concerning the escape of the secondary zoospores were given, nor is it evident that the manner of dehiscence was observed.

Butler (2), however, gave a more satisfactory illustrated account of the diplanetic development manifested by his *Pythium diacarpum*. His description is confirmed by the writer's own observations on diplanetism in a number of species of *Pythium*, including, for example, *P. butleri* Sub., the parasite often associated with decay of various commercial cucurbitaceous fruits and of snap beans (*Phaseolus vulgaris* L.) in transit and on the market. The somewhat large primary zoospore of this fungus, after encystment measuring mostly 10 or 11 μ in diameter, produces an evacuation tube usually 1.9 to 2.2 μ in diameter and 3 to 25 μ in length, the protoplasmic contents in the meantime displaying a vacuole of increasing size. Finally the refringent tip of the evacuation tube yields and the contents flow out to collect at the orifice, the streaming requiring usually from 30 to 45 seconds. The discharged protoplast soon begins to exhibit writhing movements, which, as cilia make their appearance and a grooved reniform shape is assumed, become increasingly energetic though restricted within a confined space. Usually about 20 minutes after the cessation of streaming the now violently active zoospore dashes away as a free-swimming body. The restriction in amplitude of movement of the secondary spore previous to its ultimate liberation is interpreted as evidence of the presence of a confining vesicle. Direct though somewhat dubious visible evidence of such a vesicle is provided now and then in indications of an approximately circular contour coinciding with the limits of the field of movement and never more than faintly discernible even in proximity to the mouth of the evacuation tube. Since in preparations of the same fungus the vesicles associated with the smallest sporangia of vegetative origin, producing 2, 3, or 4 zoospores, are often nearly if not quite as difficult to make out, the parallelism between the production of primary and of secondary zoospores is, indeed, little less than complete. While *Pythium butleri* represents a species with lobulate sporangia, similar diplanetic development was observed in extraordinary abundance in a congeneric form with undifferentiated filamentous sporangia isolated from diseased roots of sugarcane (*Saccharum officinarum* L.).

Cornu (4) mentioned *Pythium proliferum* De Bary and its varieties as examples of saprolegniaceous forms, the zoospores of which may emit zoospores similar to themselves as an alternative to putting forth vegetative germ tubes. The details of the repetitional develop-

ment were not discussed further. Cornu described development of zoospores in *P. proliferum* as taking place often within a vesicle formed at the extremity of the sporangium and at other times within the sporangium itself, the ready-fashioned zoospores in the latter case escaping directly when discharge occurred, since, although a vesicle appeared, it persisted only a few instants. The departure from the course of development generally prevalent in the genus *Pythium* is interesting when considered in connection with Butler's statement that in some cases in *P. diacarpum* much of the sporangial contents becomes divided at the mouth of an urn-shaped modification in the absence of a vesicle.

A more striking parallel with the condition set forth by Cornu is provided in the literature dealing with *Pythiomorpha gonapodioides* Petersen. According to the accounts of Petersen (18), Minden (15), and Kanouse (14), the fashioning and discharge of zoospores in the latter fungus would seem to be similar to that among species of *Phytophthora*. The vesicle described by Minden is evidently an ephemeral, poorly developed structure, bursting at an early stage in the evacuation of the sporangium to allow the completely formed zoospores to swim away without much delay—not differing greatly, therefore, from the bladderlike membrane noted by Dastur (5) in *Phytophthora parasitica*, and by Rosenbaum (21) in *P. cactorum* and *P. arecae* (Colem.) Pethyb. In Kanouse's account a second swarming was mentioned, the protoplasm passing slowly out of the cyst wall, though cilia were not observed, and no activity of the liberated naked cell other than the emission of a germ tube was represented in the accompanying illustration.

Because of the dearth of detail concerning the development of locomotive organs, it is not possible to refer the diplanetism development in question either to the type prevailing in the genus *Phytophthora* or in *Pythium*. In any case the mere presence of such development can hardly be advanced as a strong argument in favor of the distinctiveness of any form or group from either of these genera.

Although a fungus received in artificial culture from the Centraalbureau voor Schimmelcultures as having been contributed originally by Kanouse under the name *Pythiomorpha gonapodioides* has yielded no structures relating to diplanetism in the few irrigated preparations examined by the writer, a number of undoubtedly closely related species have revealed such structures in moderate abundance. Of these species, one isolated from diseased rootlets of ragweed (*Ambrosia trifida* L.) and referred to in a brief abstract (8) manifestly gives rise to the secondary swimming stage, not only by the fashioning of the motile body within the cyst wall and its direct delivery through an evacuation tube, but also indirectly by the production of miniature sporangia. The former mode of development is shown by the presence of empty spherical cyst envelopes usually measuring 11 to 16 μ in diameter, each provided with an open evacuation tube 3 to 6 μ in diameter and 5 to 15 μ in length. When miniature sporangia are produced they are borne on germ sporangiophores mostly 2 μ in diameter and ranging from 40 to 285 μ in length. Frequently an empty cyst envelope with an open evacuation tube is provided with one or two narrower filamentous outgrowths suggesting abortive germ sporangiophores. Although unfortunately the fashioning and liberation of the secondary motile

bodies were not observed, the occurrence of two types of development paralleling those observed in various species of *Phytophthora* is not without interest.

However little tenable ground the writings on *Pythiomorpha* offer for the maintenance of that genus as a separate taxonomic entity, the fungus discussed by Kanouse as *P. gonapodioides* does not seem referable to *Phytophthora*. In the hands of the present writer the presumably identical organism never produced zoospores within the sporangium to discharge them full fledged through an orifice provided by a uniformly sessile papilla. On the contrary, development took place as in typical representatives of *Pythium*, by the discharge of undifferentiated contents into a vesicle in which division into zoospores was accomplished in 15 or 20 minutes. The papilla of dehiscence was sometimes sessile and at other times surmounted an evacuation tube 5μ or more in length. In versatility of asexual reproduction the fungus thus recalls Cornu's early description of similar behavior in *Pythium proliferum*. A larger measure of distinctiveness is embodied apparently in the somewhat unusual shape of the antheridium and its manner of application to the oogonium. In Kanouse's drawing of the sexual apparatus, the male organ is represented as an elongated clasping structure evidently applied lengthwise to the oogonium. A similar shape and manner of application is shown in Dissmann's figures (6, figs. 19 and 33) of the antheridium of his *Pythium proliferum*. As far as the writer is aware, no antheridium of similar type and of similar disposition with reference to the female structure has been recorded for any fungus definitely assigned to *Phytophthora*.

SUMMARY

In many species of *Phytophthora*, the zoospores after encystment give rise to secondary swimming spores similar to themselves except for a slight inferiority in size. The repetitional development follows either one of two courses. A papilla or tube of dehiscence, usually relatively broad and of variable length, may be produced, which on yielding at the apex permits the immediate escape of the completely formed secondary zoospore fashioned in the meantime from the protoplast with accompanying writhing movements visible in retractions here and there from the cyst wall. Or a slender but often rather long germ sporangiophore, terminating in a miniature papillate sporangium delimited by a basal septum, may be put forth, either from the periphery of the cyst or from an unsuccessful evacuation tube. The contents of the miniature sporangium are eventually discharged from the papillary orifice as a completely developed motile zoospore.

Only development of the more direct type was observed in *Phytophthora fagi* and in three unnamed congeneric fungi with non-papillate, proliferous sporangia, one originating from a diseased Idaho potato tuber, one from a mature sugar-beet root in Utah, and the other from a decaying tomato fruit in California. In *P. hibernalis* only the second indirect type of repetitional development was noted. Both types were found in all strains referable to *P. parasitica* in a somewhat broad sense, including *P. parasitica* var. *rhei*, and possibly a papillate form isolated from decaying potato tubers. *P. cactorum*, *P. citrophthora*, *P. melongenae* and a fungus

previously reported as having been isolated from a diseased Honey-dew melon represent other congeneric parasites exhibiting direct as well as indirect diplanetism.

When dehiscence of the ordinary sporangia is partly or wholly frustrated, the imprisoned zoospores after encystment often give rise to a free-swimming stage through repetitional development. The germ sporangiophores perforating the sporangial wall or passing out through the papillary orifice to bear the miniature sporangia externally often lend a distinctive bristling aspect to the resulting reproductive arrangement.

Diplanetism was observed in various species of *Pythium* with filaments and with lobulate sporangia, as, for example, *P. butleri*, the encysted structure discharging its undifferentiated contents through a slender evacuation tube into a small vesicle where they are fashioned into a single motile spore. In *Pythium* as in *Phytophthora*, therefore, repetitional development follows the course generally characteristic of zoospore formation in the genus. In certain allied forms distinguished by elongated clasping antheridia closely applied lengthwise to the oogonium, and by mostly terminal and often proliferous sporangia, the occurrence of empty cyst envelopes and of germ sporangiophores terminating in miniature sporangia provide proof of the prevalence of diplanetic development.

LITERATURE CITED

- (1) ASHBY, S. F.
1922. OOSPORES IN CULTURES OF PHYTOPHTHORA FABERI. Roy. Bot. Gard. Kew, Bul. Misc. Inform. 1922: [257]-262.
- (2) BUTLER, E. J.
1907. AN ACCOUNT OF THE GENUS PYTHIUM AND SOME CHYTRIDIACEAE. India Dept. Agr. Mem., Bot. Ser. 1: 1-161, illus.
- (3) CARNE, W. M.
1927. A BROWN ROT OF CITRUS IN AUSTRALIA (PHYTOPHTHORA HIBERNALIS N. SP.). Jour. Roy. Soc. West. Aust. 12: 13-41, illus.
- (4) CORNU, M.
1872. MONOGRAPHIE DES SAPROLÉGNIEES, ÉTUDE PHYSIOLOGIQUE ET SYSTÉMATIQUE. Ann. Sci. Nat., Bot. (5) 15: [5]-198, illus.
- (5) DASTUR, J. F.
1913. ON PHYTOPHTHORA PARASITICA NOV. SPEC., A NEW DISEASE OF THE CASTOR OIL PLANT. India Dept. Agr. Mem., Bot. Ser. 5: [177]-231, illus.
- (6) DISSMANN, E.
1927. VERGLEICHENDE STUDIEN ZUR BIOLOGIE UND SYSTEMATIK ZWEIER PYTHIUMARTEN. Arch. Protistenk. 60: 142-192, illus.
- (7) DRECHSLER, C.
1926. FOOT-ROT OF LILIUM CANDIDUM AND LILIUM PYRENAICUM CAUSED BY PHYTOPHTHORA CACTORUM. Phytopathology 16: 51-53.
- (8) ———
1927. A PECULIAR TYPE OF PYTHIUM. (Abstract) Phytopathology 17: 55.
- (9) ———
1929. A FRUIT ROT OF HONEY DEW MELONS DUE TO A SPECIES OF PHYTOPHTHORA. (Abstract) Phytopathology 19: 85.
- (10) ———
1929. A DIPLANETIC SPECIES OF PHYTOPHTHORA CAUSING PINK ROT OF POTATO TUBERS. (Abstract) Phytopathology 19: 92.
- (11) GÄUMANN, E. A.
1928. COMPARATIVE MORPHOLOGY OF FUNGI. Transl. and rev. by C. W. Dodge. 701 p., illus. New York and London.
- (12) GODFREY, G. H.
1923. A PHYTOPHTHORA FOOTROT OF RHUBARB. Jour. Agr. Research 23: 1-26, illus.

- (13) HOTSON, J. W., and HARTGE, L.
1923. A DISEASE OF TOMATO CAUSED BY PHYTOPHTHORA MEXICANA, SP. NOV. *Phytopathology* 13: [520]-531, illus.
- (14) KANOUSE, B. B.
1925. PHYSIOLOGY AND MORPHOLOGY OF PYTHIOMORPHA GONAPODIOIDES. *Bot. Gaz.* 79: [196]-206, illus.
- (15) MINDEN, M. VON.
1916. BEITRÄGE ZUR BIOLOGIE UND SYSTEMATIK EINHEIMISCHER SUBMERSEER PHYCOMYCETEN. In Falck, R. *Mykologische Untersuchungen und Berichte* 1: 146-255, illus.
- (16) MURPHY, P. A.
1920-22. THE BIONOMICS OF THE CONIDIA OF PHYTOPHTHORA INFESTANS (MONT.) DE BARY. *Roy. Dublin Soc. Sci. Proc. (n. s.)* 16: 442-466.
- (17) OCFEMIA, G. O.
1925. THE PHYTOPHTHORA DISEASE OF EGGPLANT IN THE PHILIPPINE ISLANDS. *Philippine Agr.* 14: 317-328, illus.
- (18) PETERSEN, H. E.
1910. AN ACCOUNT OF DANISH FRESHWATER-PHYCOMYCETES, WITH BIOLOGICAL AND SYSTEMATICAL REMARKS. *Ann. Mycol.* 8: [494]-560, illus.
- (19) RACIBORSKI, M.
1892. PYTHIUM DICTYOSPORUM, NIEZNANY PASCRZYT SKRETNICY. (PYTHIUM DICTYOSPORUM, EIN NEUER PARASIT DER SPIROGYRA.) *Bul. Internatl. Acad. Sci. Cracovie* 1891: 283-287.
- (20) REDDICK, D.
1920. A FOURTH PHYTOPHTHORA DISEASE OF TOMATO. *Phytopathology* 10: [528]-534.
- (21) ROSENBAUM, J.
1917. STUDIES OF THE GENUS PHYTOPHTHORA. *Jour. Agr. Research* 8: 233-276, illus.
- (22) SAWADA, K.
1927. DESCRIPTIVE CATALOGUE OF THE FORMOSAN FUNGI. PART III. Japan Dept. Agr., Gov. Research Inst. Formosa, Rpt. 27, 62 and 7 p., illus. [In Japanese, English translation on file in Bureau of Plant Industry.]



EFFECTS OF DEFOLIATION AND ROOT PRUNING ON THE CHEMICAL COMPOSITION OF SWEET-CORN KERNELS¹

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INTRODUCTION

Since sweet corn in the fresh and in the canned condition is widely used for human food, it is desirable to know as much as possible of the relation of cultural practices and crop hazards to its composition and quality. In the field the corn plant is often mutilated in various ways; the root system may be injured by the plow or the cultivator; windstorms may throw the plants to the ground; hail may tear the leaves and greatly interfere with their normal functions; and insects may consume more or less of the foliage and injure the stalks. Theoretically, these conditions which directly affect the nutrition and development of the corn plant should affect the composition and quality of the grains. The object of the present work was to determine whether or not such is the case.

LITERATURE

Heckel (7, 8, 9, 10),² in a series of studies on the effect of emasculation on the sugar content of corn and sorghum, found that in general this treatment resulted at first in an increase in the amount of saccharose, levulose, and dextrose over that of the check plants, followed by a decrease in these sugars. Some variation was noted, however, in the behavior of different varieties. This worker made a study of the possible cumulative effect of traumatism on the sugar content in the progeny of corn detasseled for four successive years. An increase in total saccharose and glucose over that in the control plots was noted, the greatest percentage total increase being obtained from strains previously showing only moderate sweetness and the maximum being attained in 24 days after detasseling. No data were given on the chemical composition of the kernels in any of the work above cited.

Haller and Magness (6) investigated the relation of leaf area to the growth and composition of apples, and their work is mentioned here as indicating the importance of the amount of foliage in determining the nature of the crop. Apples grown with a large leaf area were higher in dry weight, sugar, and acids than those with smaller leaf surface, and the quality of the fruit was superior. Ripening took place more promptly.

Dungan (4) reported on the influence of plant injury and root-rot diseases on the physical and chemical composition of corn grains. In one series of experiments the stalks and shanks of ears were broken but not severed when the grain was in the milk stage and in another series when the grain was in the soft-dough stage. In other experiments the corn seeds were inoculated with root-rot organisms, and the

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² Reference is made by number (italic) to "Literature cited," p. 582.

effect on the composition of the grain of plants derived from this seed was determined. The effect of breaking the stalks and shanks of ears was the same on the yield and composition of the grain as premature harvesting. Breaking the shanks when the ears were in the soft-dough stage caused the greatest chaffiness in the grain. Chemical analyses showed no definite correlation between the apparent starchiness of the kernel and the actual quantity of starch. The percentage of total nitrogen, hemicellulose, and nonhydrolyzable material was distinctly higher in grain from ears produced on broken shanks than in grain from ears produced on sound shanks. The mutilation of the shanks resulted in a lower ether extract and starch content in the grain. Inoculation of the seed at planting time with root-rot organisms resulted in grain of a lower specific gravity than that from the uninoculated seed but no significant differences were noted in the chemical composition of the grain produced. There was slightly more nitrogen in the corn from inoculated seed and a little less ether extract and total sugar.

METHOD OF INVESTIGATION

The variety of corn used in the present investigation was Stowell Evergreen, grown at the Arlington Experiment Farm, Rosslyn, Va., during the season of 1927. The soil was a deep, fertile loam, and the seeding was heavy. When the plants were 8 to 12 inches high they were thinned, vigorous plants being left at 14 to 18 inches in the row. The rows were 3.3 feet apart. The plants used in the investigation were from two plots, one of which was planted on June 22 and the other on June 27. The combined plots, which were contiguous, totaled one-fifth of an acre. They were inspected daily from August 19 to September 10, and, for later identification, the newly forming ears were tagged on the day of the first appearance of silks, as in earlier studies (2, 3, 11). The plants on which the present report is based, between 400 and 500 in number, silked during the period from August 26 to August 31. They were treated as 10 separate groups, though the individual plants of each group were uniformly distributed within the two plots. The groups were treated as follows:

Group 1.—The leaf at the ear was left and the alternate leaves, both above and below the ear were severed close to the stalk. This was done on the day of silking.

Group 2.—All leaves except the one at the ear were removed on the date of silking.

Group 3.—All leaves were removed five days after silking.

Group 4.—Treated in the same manner as group 3, except that defoliation was performed 10 days after silking.

Group 5.—The leaves were all removed 15 days after silking.

Group 6.—The leaves were all removed 20 days after silking.

Group 7.—The plants were assumed to stand in the center of a square 6 inches on each side, and by means of a sharp spade the roots were cut to a depth of 18 inches on two adjacent sides of the square. It was assumed that one-half the roots were pruned away by this treatment. This was done on the date of silking.

Group 8.—The roots were pruned away to a depth of 18 inches on all four sides of the 6-inch square on the day of silking.

Group 9.—Partial defoliation and partial destruction of roots were performed, half the leaves and half the roots being destroyed on the day of silking.

Group 10.—Entirely normal, representative plants which received the usual cultivation and were not mutilated in any way served as controls.

Pressure of other work prevented a further elaboration of the root-pruning experiments.

None of these plants produced suckers or showed regeneration of leaves following defoliation.

At intervals of 5 days up to 30 days after mutilation, representative samples of 8 to 12 ears were collected where feasible from each group. The ears were taken to the laboratory, husked and silked and the kernels cut from the ear with a sharp knife, close to the cob. The material thus obtained was then thoroughly mixed and duplicate samples of 100 gm. each weighed out, covered at once with 95 per cent alcohol, and heated to boiling to inactivate the enzymes and prevent chemical changes. At the end of the season these samples were analyzed for carbohydrates, following the method of the Association of Official Agricultural Chemists (1).

During the period of the experiment the weather conditions were very favorable for corn. There was a sufficiency of rain, with normal summer temperatures and an absence of heavy winds or violent storms that might have injured the plants or interfered with the progress of the experiments.

The size of the ears and the yields of corn were not carefully determined, but a fair estimate was made by comparing the ears and yields of the mutilated plants with those from the normal controls.

EXPERIMENTAL RESULTS

The chemical data obtained from the analysis of samples from the different groups of plants are presented in Table 1, and a statistical analysis of the differences in total solids, total sugars, and acid-hydrolyzable substances between the various test samples and the controls is shown in Table 2. Use here was made of the symbols and formulas employed by Fisher (5) in testing the significance of the mean of small samples. In the headings of the various columns, \bar{y} represents the mean of the small sample, s the standard deviation of this sample, and $\frac{P}{2}$ the probability of the value t derived by use of the formula $t = \frac{\bar{y} - \bar{y}'}{s}$, or similar formula suited to the treatment of allied data, being exceeded in a positive or negative direction only, in other samples from the same universe. In the use of this symbol, only those values of $\frac{P}{2}$ less than 0.050 are considered to indicate significant differences; the smaller the value of $\frac{P}{2}$ the more significant the difference. A brief consideration of the formulas used in the present calculations is to be found in the footnotes to Table 2. The minus and plus signs in the \bar{y} columns show respectively whether the mean of the total solids, total sugar, and acid-hydrolyzable substances of the test corn at different stages of development was less or greater than the mean of the control corn sampled at like stages of development.

TABLE 1.—*Effect of defoliation and root pruning on the chemical composition of the developing grains of Stowell Evergreen sweet corn*

Group No.	Treatment of plants	Age of ears from date of silking	Total solids	Alcoholic extract	Residue	Total sugar	Reducing sugar	Sucrose	Acid-hydrolyzable substances
		Days	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	Half of leaves removed at time of silking.....	10	9.67	6.13	3.54	4.24	2.94	1.30	1.45
		15	13.83	8.02	5.81	5.78	2.30	3.48	3.25
		20	18.05	7.62	10.43	4.87	1.49	3.38	8.12
		25	22.46	7.40	15.06	3.98	.96	3.02	12.01
		30	25.97	6.36	19.61	3.43	.62	2.81	16.08
2	Leaves all removed except one at time of silking.....	10	8.87	5.60	3.27	3.92	2.15	1.77	1.30
		15	11.60	6.60	5.00	3.92	2.09	1.83	2.75
		20	17.79	7.24	10.55	5.16	1.00	4.16	5.80
		25	22.37	7.01	15.36	4.83	.87	3.96	12.60
		30	23.08	6.05	17.03	4.47	.78	3.69	14.13
3	Leaves all removed 5 days after silking.....	10	9.25	5.84	3.41	3.31	2.50	.81	.79
		15							
		20	14.21	6.48	7.73	4.36	1.28	3.08	3.86
		25	22.37	6.81	15.56	4.02	.83	3.19	10.26
		30							
4	Leaves all removed 10 days after silking.....	10							
		15	11.80	7.41	4.39	5.02	2.00	3.02	2.56
		20	15.46	6.76	8.70	4.64	1.23	3.41	5.22
		25	22.90	6.82	16.08	3.70	.75	2.95	11.90
		30	18.45	5.29	13.16	1.93	.79	1.14	7.96
5	Leaves all removed 15 days after silking.....	10	26.78	0.40	20.38	3.01	.56	2.45	14.67
		15	19.45	4.84	14.61	1.79	.55	1.24	9.01
		20							
		25	14.46	6.20	8.26	3.33	1.04	2.29	5.04
		30	21.63	5.48	16.15	2.04	.46	1.58	11.62
6	Leaves all removed 20 days after silking.....	10	25.34	4.91	20.43	1.84	.39	1.45	14.71
		15							
		20	26.92	4.16	22.76	1.88	.47	1.41	16.61
		25	11.12	7.12	4.00	4.16	2.20	1.96	1.64
		30	15.88	8.56	7.32	5.76	2.07	3.69	4.03
7	Roots pruned on 2 sides at time of silking.....	10	20.52	8.18	12.34	5.04	1.29	3.75	8.74
		15	24.46	7.08	17.38	4.01	1.01	3.00	14.25
		20	27.31	6.54	20.77	3.63	.44	3.19	17.23
		25							
		30	15.78	8.36	7.42	5.73	1.74	3.99	4.08
8	Roots pruned on 4 sides at time of silking.....	10	20.70	8.04	12.66	5.02	1.17	3.85	8.99
		15	27.70	7.84	19.86	4.46	.76	3.70	16.28
		20	31.30	5.88	25.42	3.23	.71	2.52	21.1
		25	9.68	6.06	3.57	3.25	1.83	1.42	1.43
		30	14.70	8.02	6.68	4.90	1.90	3.00	3.61
9	Half of leaves removed and half of roots cut at time of silking.....	10	18.86	7.80	11.06	4.94	1.01	3.93	7.85
		15							
		20	29.45	6.14	23.31	3.02	.58	2.44	19.34
		25	9.96	6.00	3.96	4.06	2.88	1.18	1.61
		30	14.44	7.60	6.84	5.76	1.84	3.92	3.76
10	No treatment (used as control).....	10	18.82	7.16	11.66	4.84	1.36	3.48	8.35
		15	23.17	6.72	16.45	4.17	.74	3.43	12.78
		20	25.45	5.94	19.51	3.37	.62	2.75	16.19
		25							
		30							

¹ Normal grains.² Shriveled grains.

TABLE 2.—*Significance of differences in total solids, total sugars, and acid-hydrolyzable substances between corn from defoliated and root-pruned plants and that from untreated controls, as determined by statistical analysis*

[Calculated from the data of Table 1]

Group No.	Total solids			Total sugars			Acid-hydrolyzable substances		
	\bar{y}	s	$\frac{P}{2}$	\bar{y}	s	$\frac{P}{2}$	\bar{y}	s	$\frac{P}{2}$
1.....	-0.372	0.531	0.097	+0.020	0.134	0.378	-0.356	0.278	0.024
2.....	-1.626	.915	.009	+0.020	1.134	(¹)	-1.222	1.052	.032
3.....	-2.040	2.226	.130	-.460	.300	.063	-2.610	1.836	.072
4.....	-1.235	2.160	.169	-.443	.227	.017	-1.883	.999	.023
5.....	-2.003	² .564	(⁴)	-1.723	.352	.007	-1.983	1.160	.049
6.....									
7.....	+1.490	.288	(¹)	+0.080	.167	.174	+0.640	³ .362	(⁴)
8.....	+3.400	⁴ .624	(⁴)	+0.075	1.953	(¹)	+2.362	³ .728	(⁴)
9.....	+0.990	2.019	.199	-.490	.448	.085	+0.580	1.720	.275

¹ More than 0.9.² The data given under 4 are based on the results from normal-appearing grains.³ In these cases a trend with age was shown, and the data here were calculated from the line of regression. The formulas used were:

$$s = \sqrt{\frac{S(y-Y)^2}{n'-2}} \text{ and } n = n' - 2,$$

in which

 $(y-Y)$ = the deviation of y from the calculated value Y on the line of regression. \bar{a} = mean of the y 's. n' = number of items in the small sample.

In all other cases the formulas

$$s^2 = \frac{S(y-\bar{y})^2}{n'-1}, \quad t = \frac{\bar{y}-\bar{y}'}{s}, \text{ and } n = n' - 1 \text{ were used.}$$

⁴ In these cases it is not possible to express the significance of the difference between test and control by a single value. (Fisher, *ibid.*, pp. 99-123.) The formulas for the lines of regression are:(a) $y = 12.628 - 0.425x$, the range of the x 's being 20 to 30 days, inclusive.(b) $y = 3.881 + 0.324x$, the range of the x 's being 15 to 30 days, inclusive.(c) $y = -.643 + 0.064x$, the range of the x 's being 10 to 30 days, inclusive.(d) $y = -5.229 + 0.337x$, the range of the x 's being 15 to 30 days, inclusive. In all cases the interval of observation was 5 days.⁵ Less than 0.1.

EFFECT OF PARTIAL AND COMPLETE DEFOLIATION

On the whole, partial defoliation at the time of silking (group 1) made but little difference in the chemical composition of the grain as compared with that from normal plants, though the acid-hydrolyzable substances were definitely lower than in the control corn. In the case of the corn from plants entirely defoliated at the time of silking (group 2) the total solids and the acid-hydrolyzable substances were significantly lower than in the control corn.

The most marked effect of defoliation at this stage of development, as determined by careful observation, was in the size of the ears and in the yields. In the case of the plants from which half of the leaves were removed the ears were only one-half to two-thirds as large as normal ears, and on the ears of the nearly completely defoliated plants the kernels were smaller in size than on normal ears. They appeared to have developed more slowly, but in all other respects seemed normal. There was considerable variation in the behavior of the plants treated in this way at the time of silking. Some produced no seed at all, the ovules failing to develop, while others developed from 50 to 200 grains. The normal plants produced from 350 to 400 kernels to the ear.

The data showing the effect of complete defoliation five days after silking on the chemical composition of the corn (group 3) are incomplete, but so far as they are available they indicate results similar to those where the leaves were removed at the time of silking. The differences between this corn and the controls, however, were less significant, though more complete data might have shown more marked effects of this treatment. Inspection of the ears of his group showed that some kernels started to develop and subsequently failed. They had developed sufficiently, however, to be included in the sample, and although they formed but a relatively small percentage of the total sample, their effect on the composition was perceptible.

Defoliation 10 days after silking (group 4) resulted in marked physical and chemical differences in the grains. The ears from this defoliated corn sampled 15 and 20 days after silking showed some kernels that seemed to be developing normally and others that had failed completely to develop. In the corn sampled 25 and 30 days after silking the differences were so marked that two sets of samples were taken, one of the normal-appearing kernels and another of the shriveled grains. Differences in the chemical composition of these two sets of samples are shown in Table 1. The "normal" kernels had approximately the same composition as those from the plants defoliated at the time of silking. The sugar and the acid-hydrolyzable substances were significantly lower than in the kernels of the control corn. In the shriveled grains, on the other hand, the total solids and the sugar and acid-hydrolyzable substances were much lower than in the control samples. It is apparent that the incoming storage material, in the case of the ears of this lot, was insufficient to meet all needs, and a part of the kernels received it all, the remaining being left to wither.

Cessation of development did not occur at exactly the same time in all kernels that failed to mature properly; consequently, in the later stages of development the ears showed fewer normal grains than those harvested earlier. There was, of course, considerable variation in the behavior of individual plants. A small percentage of ears was found on which all the kernels appeared to have stopped developing at the same time. In such cases the plant as a whole ceased to function and usually withered and died prematurely.

The corn from plants defoliated 15 days after silking and sampled after 20, 25, and 30 days (group 5) was characterized by a significantly lower content of sugar and acid-hydrolyzable substances than the control corn.

The single sample from corn defoliated 20 days after silking and sampled 30 days later (group 6) showed higher total solids, lower sugar content, and slightly higher content of acid-hydrolyzable substances than the corn from the control plants. It is probable that these differences were due, in part at least, to the drying out of the corn prior to sampling. The data are too few, however, to warrant definite conclusions on this point.

The ears of this lot showed again the unequal development of the grains. Differences in development could not be discerned as soon after defoliation, however, as in the corn mutilated at or shortly after the silking time. There was also a larger percentage of ears on which grains failed to continue development.

EFFECT OF ROOT PRUNING

The total solids and the acid-hydrolyzable substances of the corn from which half the roots were removed (group 7) were significantly higher than those of the control corn. The differences in total sugar content were not significant. The removal of the roots from the four sides of the 6-inch square (group 8) yielded effects of the same nature, but the figures were more striking. The outstanding results of root pruning were a higher content of total solids and acid-hydrolyzable substances than that of the control corn, whereas lower total solids and a lower content of acid-hydrolyzable substances characterized the defoliated corn. The signs of the data in the \bar{y} columns of Table 2 indicate this clearly.

The removal of half the roots and half the leaves from the same plants (group 9) gave analytical results similar to those of group 8, but the size of the ears in this case was much reduced. The differences between the corn from these plants and that from the controls were not significant.

As indicated by the size of ears, reduction in yields of the root-pruned corn was marked, particularly in the case of those plants which were more severely treated; but the reduction was not so great as that resulting from defoliation.

GENERAL DISCUSSION

In considering the foregoing data it must be borne in mind that the nature and degree of the response of the corn plants to the different treatments was not, from the standpoint of the individual plants, always constant or consistent. Considerable variation in the behavior of individual plants was encountered. So far as the conditions of the experiment permitted, however, the data presented represent the group response.

In these experiments the effects of mutilation of the plant on the chemical composition of the grain were most clearly exhibited in those lots treated 10 and 15 days after silking. In the case of these plants mutilated when the kernels were in an earlier stage of development, the kernels that later failed to grow withered away before significant size was attained, and hence had no significant effect on the character of the sample. Those plants mutilated when the grains were more than 15 days of age yielded corn more closely approaching normal grain according as the treatment was longer and longer delayed.

In considering these data the climatic conditions under which the corn was grown should be held in mind. During the course of the experiments temperature and moisture conditions were very favorable for the growth and maturing of the corn. If hot winds had prevailed at any period subsequent to mutilation, the failure of kernels resulting from root pruning at least would have been greater, and higher total solids of the grain would doubtless have resulted in the case of the defoliated plants.

Yields, as shown by the size of the ears and the number of kernels, were most severely affected when the plants were defoliated at the time of silking. Partial defoliation was found to be much more disastrous to yields than partial root pruning.

In an earlier publication (3) it has been shown that quality in sweet corn is dependent on tenderness of kernel hull, the nature of the polysaccharides present, the sugar content, and other factors. Any condition exerting an unfavorable influence on the uniform composition and quality of the properly harvested corn therefore must of necessity be taken into careful consideration. From these experiments it would appear that, from the standpoint of quality of the canned product, hailstorms or other agencies destroying the foliage would be most severely felt if they occurred from 10 days to 2 weeks after the corn had flowered. The same would probably be true of drought or other unfavorable seasonal conditions.

SUMMARY

Partial or nearly complete defoliation of corn plants at the time of silking caused only small differences in the chemical composition of the developing grains. It decreased the total solids somewhat and seemed to slow down slightly the rate at which the grains matured. Root pruning done at this time resulted in corn of high total solids, and the rate of maturing seemed to be slightly increased. Mutilations at this time tremendously decreased yields, as shown by the small size of the ears and the small number of kernels. Under the conditions of these experiments defoliation was more disastrous than root pruning.

Defoliation performed after development of the kernels was well underway resulted in a very marked change in the chemical composition of the grain. The effects were most severely felt when the plants were defoliated 10 and 15 days after silking, under the climatic conditions prevailing during the period of the experiment. Yields, as indicated by the size of ears and the number of kernels, were also severely reduced under these conditions, but less severely than when defoliation was performed at the time of silking. Beyond the 15-day stage the undesirable consequences of defoliation were progressively lessened as the treatment was delayed.

When the corn plant was mutilated there was a marked difference in the behavior of the grains. Some kernels developed normally, while others on the same ear entirely failed to develop. It appeared that when the organic nutrients were insufficient for all the supply was unequally distributed, a few grains receiving all.

From a consideration of the analytical data it is concluded that any condition affecting the vegetable development of the plant adversely would affect the quality of the canned product most unfavorably if it became operative 10 to 15 days after the silking of the corn.

LITERATURE CITED

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1920. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. REVISED TO NOVEMBER 1, 1919. 417 p., illus. Washington, D. C.
- (2) CULPEPPER, C. W., and MAGOON, C. A.
1924. STUDIES UPON THE RELATIVE MERITS OF SWEET CORN VARIETIES FOR CANNING PURPOSES AND THE RELATION OF MATURITY OF CORN TO THE QUALITY OF THE CANNED PRODUCT. *Jour. Agr. Research* 28: 403-443, illus.
- (3) ——— and MAGOON, C. A.
1927. A STUDY OF THE FACTORS DETERMINING QUALITY IN SWEET CORN. *Jour. Agr. Research* 34: 413-433, illus.

- (4) DUNGAN, G. H.
1926. THE INFLUENCE OF PLANT INJURY AND THE ROOT-ROT DISEASES UPON THE PHYSICAL AND CHEMICAL COMPOSITION OF CORN GRAIN. Ill. Agr. Expt. Sta. Bul. 284, p. 255-281, illus.
- (5) FISHER, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 p., illus. Edinburgh.
- (6) HALLER, M. H., and MAGNESS, J. R.
1926. THE RELATION OF LEAF AREA TO THE GROWTH AND COMPOSITION OF APPLES. Amer. Soc. Hort. Sci. Proc. 22: 189-196.
- (7) HECKEL, E.
1912. DE L'INFLUENCE DE LA CASTRATION MÂLE, FEMELLE ET TOTALE SUR LA FORMATION DU SUCRE DANS LES TIGES DE MAÏS ET DE SORGHOSUCRÉ. Compt. Rend. Acad. Sci. [Paris] 155: 686-690.
- (8) ———
1914. SUR LA CASTRATION MÂLE DU MAÏS GÉANT DE SERBIE. Compt. Rend. Acad. Sci. [Paris] 159: 595-597.
- (9) ———
1915. CRÉATION D'UNE RACE NOUVELLE DE MAÏS GÉANT À TIGE SUCRÉE. Compt. Rend. Acad. Agr. France 1: 551-554.
- (10) ———
1915. SUR LA TRANSMISSION PAR GRAINES DES EFFETS DE LA CASTRATION DANS LES TIGES DE MAÏS. Compt. Rend. Acad. Sci. [Paris] 161: 338-340.
- (11) MAGOON, C. A., and CULPEPPER, C. W.
1926. THE RELATION OF SEASONAL FACTORS TO QUALITY IN SWEET CORN. Jour. Agr. Research 33: 1043-1072, illus.

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A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA GRAMINIS¹

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INTRODUCTION

Among the lower plants there are species with sexual reproduction in which the two cells that fuse may be borne on the same gametophyte plant. These are spoken of as homothallic. In others the two cells must be borne on separate plants of different sex or strain. In many cases there is no visible difference between the two, and on morphological grounds they can not be classified as male or female. There exist, none the less, well-defined chemical or physiological or genetic differences, such that two reproductive cells of the same plant, or two of different plants of the same sex, can not fuse. Only when two of different strains or sexes are brought together does sexual reproduction take place. Such species are heterothallic.

Data concerning heterothallism in the fungi have been accumulating rapidly in recent years. It is now known to exist in certain species of the mucors, oomycetes, ascomycetes, basidiomycetes, smuts, and rusts.

Certain species of the mucors produced zygospores rarely and under little-understood conditions. Blakeslee (5)³ in 1904 found that by bringing together strains of different origin and growing them side by side, in certain cases a rich growth of zygospores would appear along the line where the two mycelia met. Further experiments showed that all of the geographic strains of a given species that were collected could be classified into two groups. Those of one group would not reproduce sexually with other members of the same group but would with any member of the other group. The two groups were morphologically alike save for a slight difference in vegetative vigor. The more vigorous was called (+), the other (-). In homothallic species, on the other hand, a single-spore culture would produce zygospores.

In this and later papers (5, 6, 8, 9, 10, 11) Blakeslee and his associates found that a (+) race of one heterothallic species when grown

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³ Reference is made by number (italic) to "Literature cited," p. 611.

in contact with a (-) race of another species would undergo the initial stages of sexual reproduction. This so-called "imperfect hybridization" proved of value in deciding which strains were (+) and which (-), as new species came under observation. Moreover, it was found that a homothallic species would show imperfect hybridization with both (+) and (-) strains of heterothallic species. The mycelium of homothallic species is bisexual, that of heterothallic species is unisexual.

The genus *Parasitella*, which lives parasitically on other molds, was studied by Burgeff in 1924 (13) and by Satina and Blakeslee in 1926 (49). In *Parasitella simplex* Bainier, living on *Absidia glauca* Hagen, the relation is sex-limited. At the point of attack the relation of host and parasite closely resemble imperfect hybridization, and a (+) strain of the parasite lives only on a (-) strain of its host, and a (-) strain on a (+) of the host. On *Rhizopus*, on the contrary, the same strain of *Parasitella* can live on both (+) and (-) strains of the host.

Satina and Blakeslee (48, 50, 51, 52, 53) carried on a series of studies of the biochemical differences between (+) and (-) sexes in mucors. The Manoilov reaction gave 85 per cent correct determination of sex in mucors, the (+) being female and the (-) male. Several significant quantitative differences were found between (+) and (-) strains, including their ability to reduce tellurium salts and potassium permanganate.

Couch (17) in 1926 reported the existence of heterothallism in *Dictyuchus*, a water mold of the oomycetes. Several lots of material collected bore sporangia only and were later tested in crosses and proved to be either male or female. Others showed sexual reproduction, and from each of these both male and female strains were isolated. No morphological difference between isolated male and female plants was noted. Only when opposite strains were grown in contact did sexual organs form. Some strains remained neutral. One parthenogenetic strain was found which bore oogonia and eggs without antheridia. It was probably bisexual, however, for when grown with a female strain it developed antheridia, and when grown with one of the male strains the latter developed antheridia.

In the ascomycetes, Derr (21) found heterothallism in *Penicillium luteum* Zuk. Monosporous cultures differed macroscopically and chemically. They bore no fertile perithecia, although a few haploid structures without spores were formed. Two by two in certain combinations, these monosporous cultures produced normal perithecia.

Dodge (26, 27, 28) found an interesting situation in the ascomycete *Neurospora*. *Neurospora tetrasperma* Shear and Dodge produced normally in each ascus four binucleate, bisexual spores which grew and reproduced homothallically. Occasionally a smaller uninucleate, unisexual ascospore formed, which grew into a unisexual plant that could reproduce only heterothallically. *N. tetrasperma* also produced asexually a considerable percentage of unisexual conidia. Another species, *N. sitophila* (Mont.) Shear and Dodge, normally heterothallic, produced in each ascus eight uninucleate, unisexual spores. When a unisexual spore of *N. tetrasperma* was grown with a spore of *N. sitophila*, hybrid perithecia resulted. Second generation hybrids have been grown. Wilcox (61) found that in *N. sitophila*

the segregation of sex factors took place in the second nuclear division in the ascus.

Bensaude (2) in 1918 found heterothallism in *Coprinus fimetarius* L. The primary mycelium from the basidiospore was gametophytic in character. It produced oidia in abundance. An isolated monosporic mycelium remained gametophytic indefinitely, but when a (+) and a (-) mycelium were grown together, fusion took place between mycelial cells or between an oidium of one and a mycelial cell of the other, and at once sporophytic mycelium appeared with binucleate cells showing the characteristic clamp connections.

Since then a voluminous literature has grown up concerning heterothallism in the basidiomycetes. It deals predominantly with the genus *Coprinus*, which contains both heterothallic and homothallic species.

Typical of these papers is one by Hanna (32) on *Coprinus lagopus* Fr. Isolated monosporous mycelia remained gametophytic and without clamp connections. They sometimes produced imperfect haploid fruit bodies, a few of which formed spores which were all of the same sex as that of the mycelium bearing them. When monosporous mycelia from the same fruit body were paired in all possible matings, certain of the matings showed clamp connections (indicative of the sporophytic generation) and others did not. The results showed "that the spores of a single fruit body of *Coprinus lagopus*, while alike morphologically, may be divided sexually into four distinct groups regardless of where the fruit body producing the spores is obtained" (32, p. 439). The four spores from a single basidium were sometimes of two sexual groups and sometimes of four. When, however, monosporous mycelia of one geographic strain were mated with those of another strain, there was complete fertility, i. e., 100 per cent of the matings produced clamp connections, thus showing that the sexual groups in all of these fruit bodies must be different. "In the 6 fruit bodies studied * * * there have been established therefore, not 4, but 24 distinct sexual groups" (32, p. 440).

The same condition of complete interfertility, or something approximating it, of geographic races of a species has been found by Kniep (37) in *Schizophyllum commune* Fr.; by Vandendries (58, 59) in *Panaeolus campanulatus* (L. and Fr.) Quelet. and *Coprinus radians* (Desm.) Fr.; by Newton (41) in *C. rostrupianus* Hansen; by Brunswik (12) in *C. friesii* Quelet., *C. comatus* Battarra, *C. fimetarius* (L.) Fr.; and by Mounce (39) in *Fomes pinicola* (Sw.) Cooke.

Kniep (36) in 1919 proved the existence of two sexes in the sporidia of the anther smut, *Ustilago violacea* Pers. Descendants of one sporidium would not fuse with each other nor with other members of the same sex. Only when the two sexes were brought together did fusions occur. He found different physiologic forms of the smut on different host plants and learned that sporidia of one physiologic form would fuse with those of another. Goldschmidt (31) carried further the work of hybridizing physiologic forms of the anther smut and made genetic studies of the progenies.

Dickinson (22) showed that in *Ustilago levis* (K. and S.) Mag. and *U. hordei* (Pers.) K. and S. the sporidia were of two sexes. Inoculation with one sex produced no infection. Inoculating with both

produced 91 per cent of infection. Even when sporidia of one sex of *U. hordei* and of the other sex of *U. levis* were used, infection followed. Of the four first-formed sporidia from a single chlamydospore, two were found to be of one sex and two of the other. Cultural differences were found in the four strains from one chlamydospore, and it was determined that segregation of the cultural characters could take place in either of the two reduction divisions.

Stakman and Christensen (54) and Stakman, Christensen, and Hanna (55) found heterothallism in *Ustilago zeae* (Berkm.) Ung. They found many physiologic forms of the smut and noted the origin of new forms by mutation. Hanna (33), continuing the work on *U. zeae*, found that monosporidial cultures could infect the host weakly but produced no galls. When a corn plant was inoculated with two cultures of opposite sex, fusion occurred just after entry, and later galls were formed. The sporidia from one chlamydospore were of two or, less commonly, four sexual groups. Two pairs of factors at least were involved. All geographic races tested showed the same sexual groups. Mutants which appeared in monosporidial cultures showed altered sexual behavior.

Of special interest is the recent announcement by Craigie (18, 19, 20) of heterothallism in rusts. *Puccinia graminis* Pers. and *P. helianthi* Schw. have been proved experimentally to be heterothallic, and there are indications of heterothallism in *P. coronata* Cda., *P. pringsheimiana* Kleb., and *Gymnosporangium* sp. Monosporidial or simple pustules produced pycnia, but very few produced aeciospores. Of the bisporidial or compound pustules nearly half produced open aecia. When pycniospores of one sex were transferred to a monosporidial infection of opposite sex, aecia developed. In nature this transference is effected by insects which feed on the nectar. More recently Hanna (34) has found pycniospores germinating, the largest seen being 15μ long.

The existence of heterothallism and the genetic behavior of the strains of a rust are determined by greenhouse studies. Details of the history of heterothallism as it takes place in the host plant can be learned only by means of the microscope. On this account, a cytological study of the pycnia and aecia of a heterothallic rust, *Puccinia graminis*, was undertaken.

MATERIAL AND METHODS

Teliospores of *Puccinia graminis* Pers. on *Agrostis alba* L. and a supply of barberry plants were obtained from H. B. Humphrey. Teliospores on *Triticum vulgare* Vill. and *Hordeum jubatum* L. were received from W. P. Fraser. The majority of the barberry plants were grown in the greenhouse. A few were planted in the field.

Tall glass tumblers or glass vases were used in inoculating. A line was filed around the bottom of the tumbler or vase and the bottom removed. The rusted straw was soaked in rain water for an hour or two. Watch crystals or halves of small Petri dishes were filled with mud, bits of the rusted straw pressed into the mud with the rusted side exposed, and the surface sprayed with an atomizer filled with rain water. Then the tumbler was lined with one or two layers of wet paper towel, the base of the stem of the plant surrounded with wet sphagnum, the tumbler placed over it, and the mud-filled Petri

dish inverted over the top of the tumbler for a lid. Another layer of wet paper towel was folded over the top and held down with a rubber band. If the plant was small, the whole plant was covered; if larger, only the growing tip of one branch was inclosed. The whole was placed under a greenhouse bench and kept moist for 48 hours and then uncovered and replaced on top of the bench. Cages made by covering wire frames with fine-mesh "tarlatone"⁴ were placed over the plants to exclude insects.

The first flecking of the leaves appeared on the fourth to the sixth day, varying with the weather. The infections were studied and counted several times a week and greenhouse records kept.

Material was fixed daily from the time of inoculation until the infections were about 3 weeks old and at longer intervals from then until the infections and the leaves bearing them died. The fixing solutions used were Flemming's medium and weak solutions, chrom-acetic-urea mixtures, and chrom-acetic-formalin mixtures. Of these, Flemming's medium solution proved most trustworthy. The material was washed, dehydrated, and embedded in 50° paraffin. Sections were cut 10 μ thick. Three methods of staining were used—Flemming's triple stain, gentian and iodine, and safranin and methylene blue. The last proved most useful.

INVESTIGATIONS

ENTRANCE AND DEVELOPMENT OF MYCELIUM

The youngest material available for study was fixed after two days in the inoculating chamber. During that time some of the teliospores germinated and produced and released sporidia which fell upon the host, usually on the leaves, where they germinated, and the resultant rudimentary fungi entered the epidermal cells. Some of the sporidia failed to germinate, probably due to local deficiency in moisture, but they had shrunk and deteriorated to such an extent that cell contents were indistinguishable.

For uniformity and clearness all drawings are oriented in the plates as the tissues drawn are oriented in the leaf, i. e., with upper epidermis at the top of the drawing or with lower epidermis at the bottom.

The germinating sporidia enter the host by passing directly through the outer wall of epidermal cells. There are no stomata on the upper surface of barberry leaves. In Plate 1, A, the earliest stage available, entry is complete. The fungus entered through the outer epidermal wall at *a*, widened into a sacklike mass in the interior of the cell, then pushed out a hypha, *b*, toward the inner surface of the host cell. There are two nuclei in the central part. If one may judge by what occurs in *Puccinia coronata*, where earlier stages have been studied (unpublished data), the division of the original sporidial nucleus occurs before entry. A lobe of the host nucleus, *c*, lies in contact with the hypha.

This primary hypha elongates, usually curving toward the central region of the host cell. In Plate 1, B, it is a curved, sausage-shaped cell, *b-d*, still binucleate, and connected by a slender beak at *b* to

⁴ An open-woven cotton cloth somewhat in the nature of cheesecloth, and nearly as coarse as mosquito netting.

the empty, collapsed, sporidial wall, *a*. The host nucleus is in attendance at *c*.

Nuclear and cell divisions soon follow. In Plate 1, C, the discarded sporidial wall, *a*, lies near the point of entry, *b*. The hypha, *b-c*, is divided by two septa into three cells. The proximal cell, i. e., that nearest the point of entry, is binucleate, the others uninucleate. In Plate 1, D, on the contrary, it is the central cell, *b*, that contains two nuclei. This is less common. Here (pl. 1, D) the hypha has broken loose from the outer epidermal wall and lies free in the interior of the host cell.

The majority of the young rust fungi are in the 3-cell stage on the second day. Comparatively few have developed beyond it. The third day, however, brings marked progress.

The next step may be just growth in length of the primary hypha, or growth in length accompanied by the formation of branches. In Plate 1, E, drawn in outline only, the unbranched hypha is 27μ long and is composed of five cells. This is an extreme case. The type shown in Plate 1, F, is far more common. Here, while the primary hypha is still short and consists of but three cells, its first cell, *a*, has pushed out a short thumblike branch, *b*, directed toward the inner epidermal wall.

In the cell drawn in Plate 2, A, the situation is more complicated. There have been two independent entries into the same epidermal cell. In *c*, presumably the older, the primary hypha grew, became septate, formed several branches, and then deteriorated. In *a*, on the contrary, grown from a sporidium at *b*, a short, thick primary hypha has formed, consisting of four bulging, uninucleate, unbranched cells. Growth in length may have been hindered in this case by the presence of the older infection.

Ordinarily each cell of the primary hypha in turn gives rise to a branch, beginning with the oldest cell. In only one instance, so far seen, did the distal cell branch first. The branches arise on the convex side of the curved hypha at the distal end of each cell. Plate 2, B, shows this. Entry occurred at *j*, the hypha grew in a curved line to *h*, then doubled back to *e*. The oldest cell, *a*, gave rise to a branch, *b*, which passed through into the next cell, forming the intracellular hypha, *c*. The parent cell, *a*, is dead. The second cell, *d*, branched in the same fashion, forming the intracellular hypha, *f*. The original cell, *d*, is dying. In the third cell, *i*, which is still vigorous, the nucleus has divided and one daughter nucleus has passed out into a short branch at *g*. In the fourth cell the only indication of branching is a rounded knob at its distal end on the convex side, at *h*. The fifth cell is unbranched.

EXPLANATORY LEGEND FOR PLATE 1

(The teliospores of *Puccinia graminis* used in growing these infections were obtained from three different hosts. The source of each drawing is indicated by abbreviations, as follows: *Agrostis alba* = (Agr.); *Triticum vulgare* = (Trit.); *Hordeum jubatum* = (Hor.))

A.—(Hor.) Two-day infection. The fungus, *a-b*, has just entered the epidermal cell. The host nucleus is at *c*. $\times 1,130$.

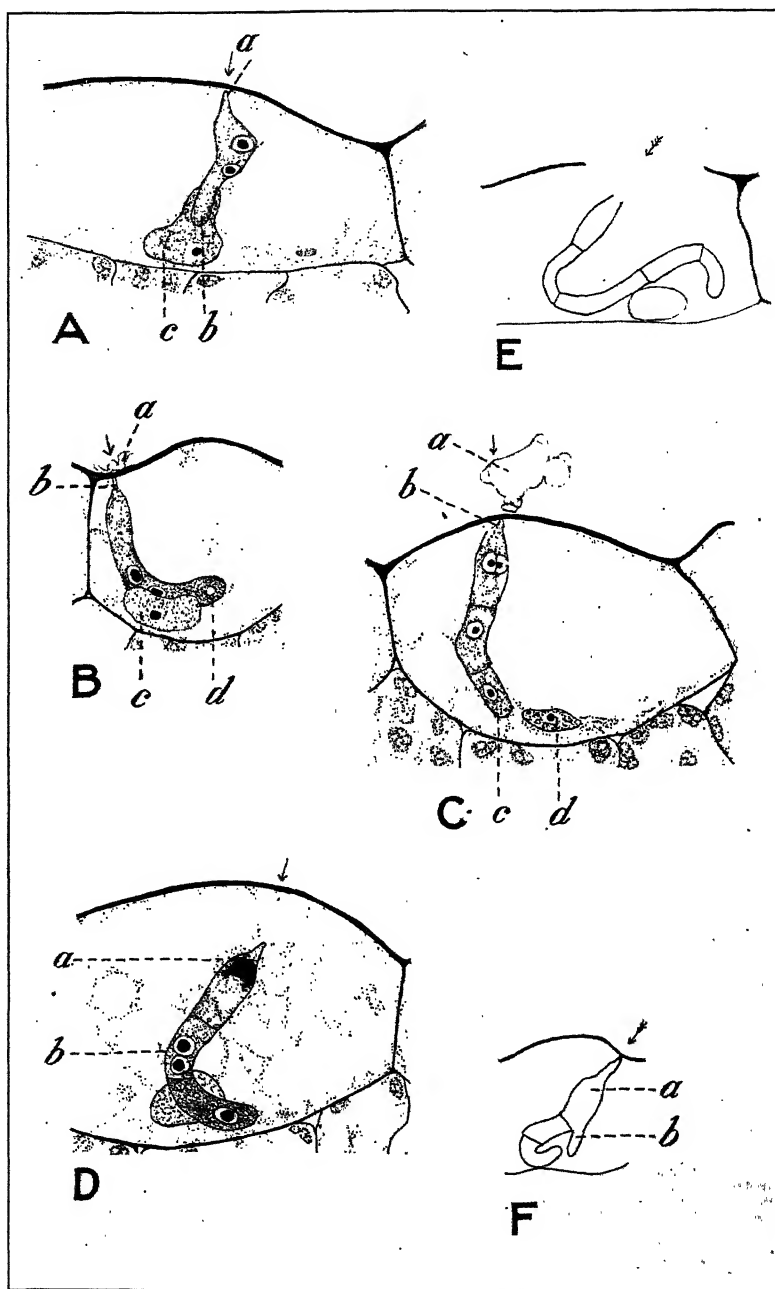
B.—(Hor.) Two-day infection. Still a single binucleate cell, *b-d*. Empty sporidial wall at *a*. Host nucleus at *c*. $\times 1,130$.

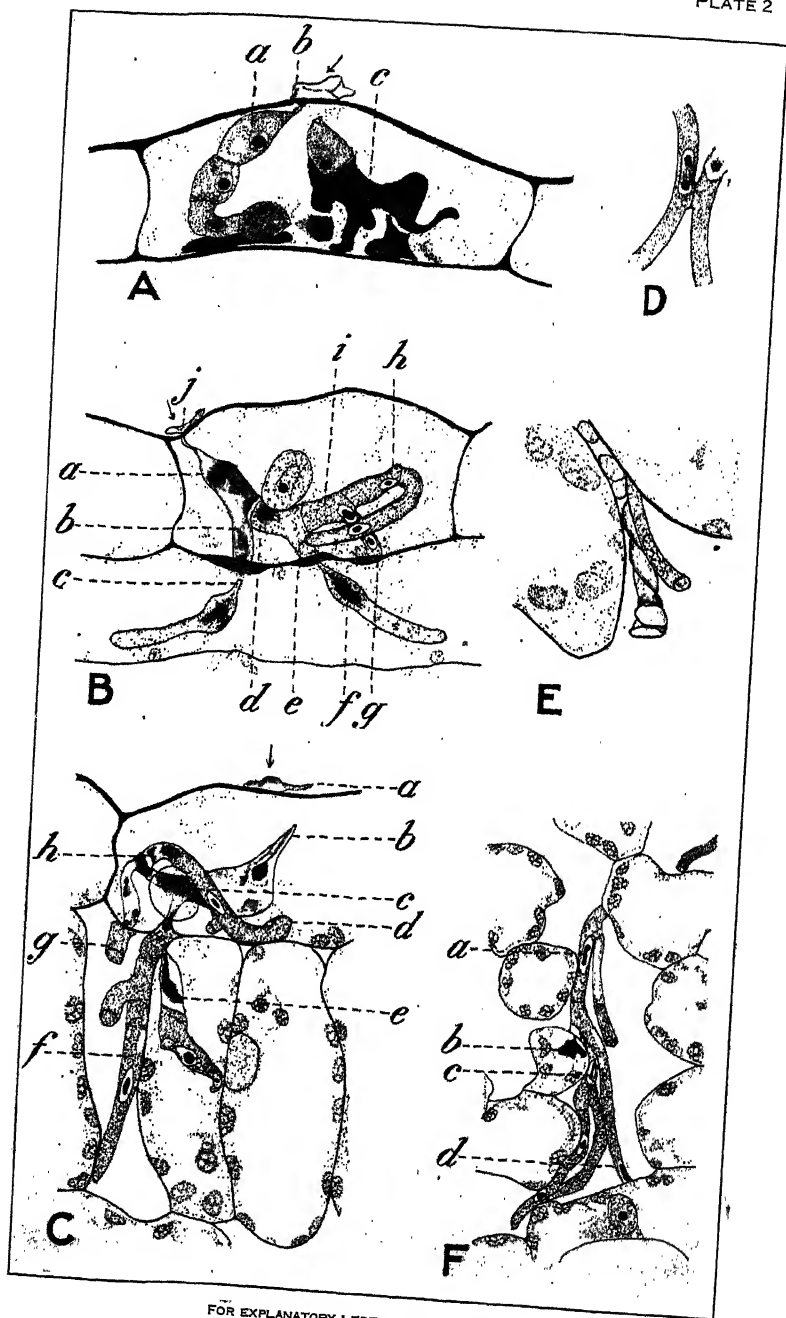
C.—(Hor.) Two-day material. Empty sporidial wall at *a*. Primary hypha, *b-c*, now 3-cell. Proximal cell binucleate. $\times 1,130$.

D.—(Hor.) Two-day infection. Three-cell primary hypha, *a-b*, free in epidermal cell. Central cell, *b*, binucleate. $\times 1,130$.

E.—(Hor.) Three-day infection. Primary hypha 5-cell, unbranched. $\times 1,130$.

F.—(Hor.) Three-day infection. Three-cell primary hypha. Proximal cell, *a*, branched at *b*. $\times 1,130$.





It is of interest that a swelling occurs in the inner epidermal wall (pl. 2, B) at each of the three points of contact with the fungus. This has not been observed elsewhere. This infection lies over a vein, and it may be that cell walls here differ chemically from palisade walls. Whether over veins or palisade cells, the actual opening in the wall through which the hypha passes is too minute to be seen.

In the infection shown in Plate 2, C, the beaklike entry tube of the hypha, *b*, lies loose in the interior of the cell, and the hypha has grown to the side wall at *h*, then doubled back to *d*, forming a loop. The host nucleus, *c*, has been left transparent in the drawing to show the fungus beneath it. All of the cells of the primary hypha have branched, and the branches have made their exit into the subepidermal area. Two of these, *f* and *g*, occur in this section, the rest in adjoining sections. Five and even six secondary hyphae may form from one primary hypha.

The rate of growth and the form of these secondary hyphae differ markedly according as they emerge into an intercellular space or into a palisade cell. The entry tube of an intracellular hypha is beaklike in shape (pl. 2, B, *c*, *f*; C, *e*; pl. 3, A, *h*), like the entry tube of the primary hypha (pl. 2, B, *a*; C, *b*; pl. 3, B, *a*). Hyphae passing into intercellular spaces, on the contrary, broaden at once to full size (pl. 2, C, *g*; pl. 3, B, *d*; pl. 3, A, *b*). The first intercellular hyphae (pl. 2, C, *f*, *g*) are vigorous and shapely and quite like the later mycelium. The growth within palisade cells, on the contrary, presents all gradations from simple haustoria to 3-cell or 4-cell hyphae not unlike the primary hypha in character (pl. 2, C, *e*). The simplest are 1-cell, unbranched, and uninucleate; others have the tips forked and curved (pl. 3, A, *c*); and some are septate and have two or three cells. The latter, like the primary hypha, are often irregular in form and are usually short-lived. A few are fully mycelial in character and grow directly through palisade cells and out into the air spaces of the mesophyll.

Once free of the compact palisade layer, the mycelium is regularly intercellular in character. Later haustoria (pl. 3, A, *h*, *i*) may be either branched or unbranched and are commonly, but not always, 1-cell.

Plate 3, B, still from 3-day material, shows another case of two primary hyphae within the same host cell. It is not rare to find four or five within a range of two or three epidermal cells. On the basis of heterothallism, a rust plant would be either (+) or (-). It seems highly unlikely that all the members of a group of four or five would be (+) or all (-). The question at once arises as to whether a (+) and a (-) hypha can fuse, giving rise directly to sporophytic mycelium. The search for this has yielded nothing conclusive. In one case (pl. 2, D) in the margin of an older mycelium, two hyphae

EXPLANATORY LEGEND FOR PLATE 2

A.—(Hor.) Three-day infection. Two primary hyphae, *a* and *c*, in one epidermal cell. Older one, *c*, dying. $\times 1,130$.

B.—(Hor.) Three-day infection. Empty sporidial wall at *f*. The primary hypha grew from *a* to *h* and back to *e*. Proximal cell, *a*, branched at *b*, forming subepidermal hypha, *c*. Second cell, *d*, formed hypha at *f*. Third cell, *i*, formed branch at *g*. $\times 1,130$.

C.—(Agr.) Three-day infection. Sporidial wall at *a*. The primary hypha grew from *b* to *h*, then back to *d*. Host nucleus at *c*. Branches formed intercellular hyphae at *f* and *g* and an intracellular hypha at *e*. $\times 1,130$.

D.—(Agr.) Detail from a 10-day infection. Two hyphae anastomosed. $\times 1,400$.

E.—(Agr.) From a 10-day infection. Two hyphae twisted around each other. $\times 1,400$.

F.—(Agr.) Detail from 4-day infection. Cells uninucleate. Elongated nuclei at *a*, *c*, *d*. Haustorium at *b*. $\times 730$.

met and seemingly anastomosed, but there was no proof of transfer of nuclei. Several times two hyphae have been found twisted tightly into a rope (pl. 2, E), but here, too, there is no proof that binucleate mycelium arises from it. So far as noted, the cells of young interlacing mycelia are strictly uninucleate, and even in older mycelia there is at present no unmistakable proof that the sporophytic mycelium has arisen directly by fusion of gametophytic hyphae.

Plate 3, B, illustrates another point. One branch, *c*, from a primary hypha, has emerged upon the outer surface of the leaf and formed a considerable growth there. Two other similar instances have been seen. The extreme humidity of the inoculation chamber probably facilitates this. It is doubtful whether it occurs often in nature.

Once the mycelium has reached the spongy mesophyll of the leaf, it spreads freely. The rate of development varies with conditions. Early in May, 4-day infections in the greenhouse consist of vegetative mycelium. In June, 4-day infections have begun reproductive activities and bear all stages up to half-grown pycnia. The difference may be due to the increased light and higher temperature at the later date.

Plate 3, A, represents the central section through a 4-day infection fixed on May 1. The remnants of the rust in the epidermis are too disintegrated to permit an interpretation. It is not certain whether a single primary hypha, *d*, passed through the side wall of the cell at *f* and formed a growth in the adjoining epidermal cell, or whether there were two independent primary hyphae. The former seems more likely. In the first palisade cell entered, the rust formed a several-cell hypha, *c*, which is dead, and its host cell is also shrunken and dying. The adjoining palisade cell was entered near *a* (the connection was lost in sectioning), the hypha grew to the inner end of the cell, branching on the way, and passed out at *b*, into intercellular spaces. From here, and from *e*, where the fungus passed directly from the epidermis into intercellular spaces, the mycelium has spread through the air spaces, covering an area 140μ in diameter. The older intercellular hyphae, at *b* and *g*, are drained of stainable content, the materials having presumably passed on toward the growing tips, *j*, *k*.

The cells of the hyphae are uninucleate so far as noted (pl. 3, A, *j*, *k*; pl. 2, F). When hyphal cells are growing rapidly, both the nucleus as a whole and the nucleolus within it may be elongated. (Pl. 2, F, *a*, *c*, *d*.)

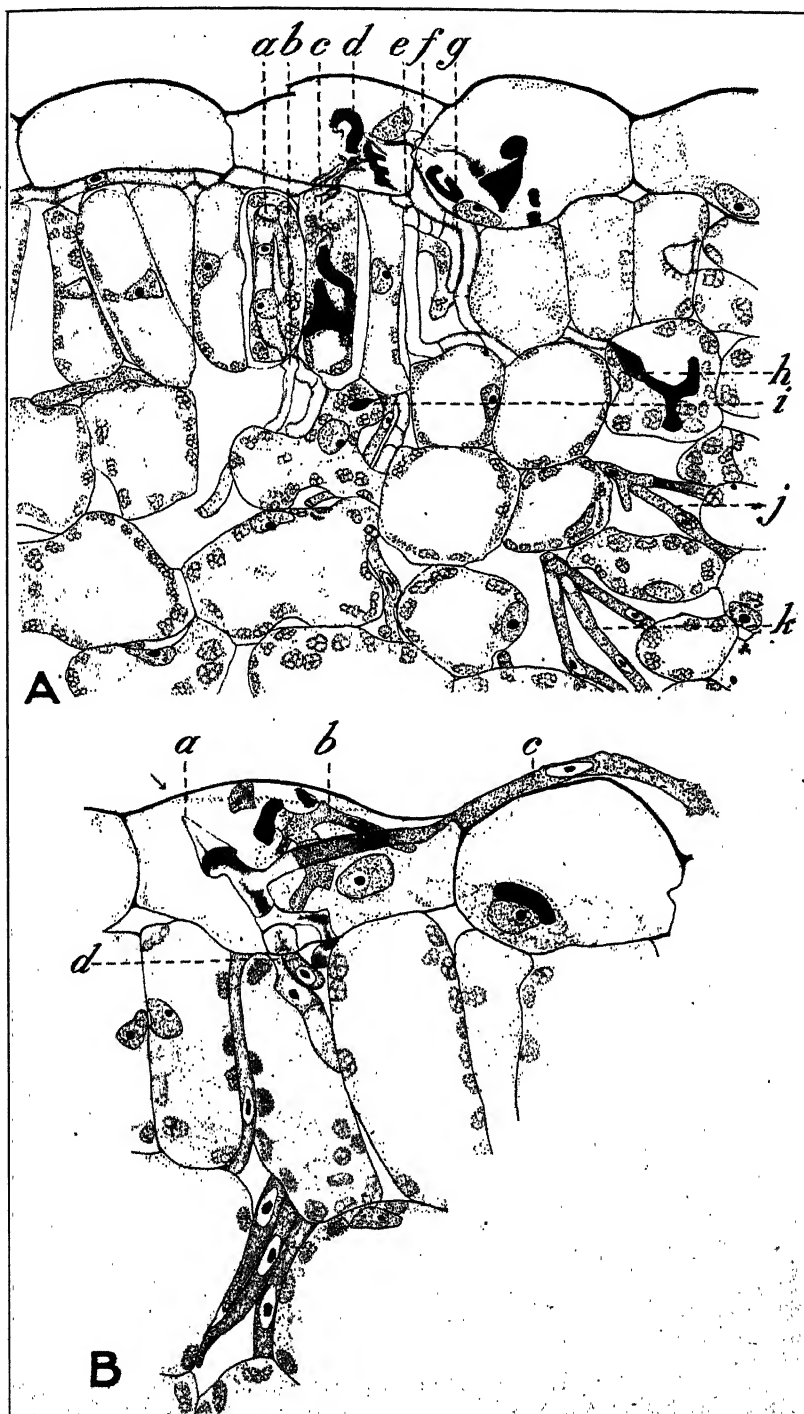
DEVELOPMENT OF THE PYCNium

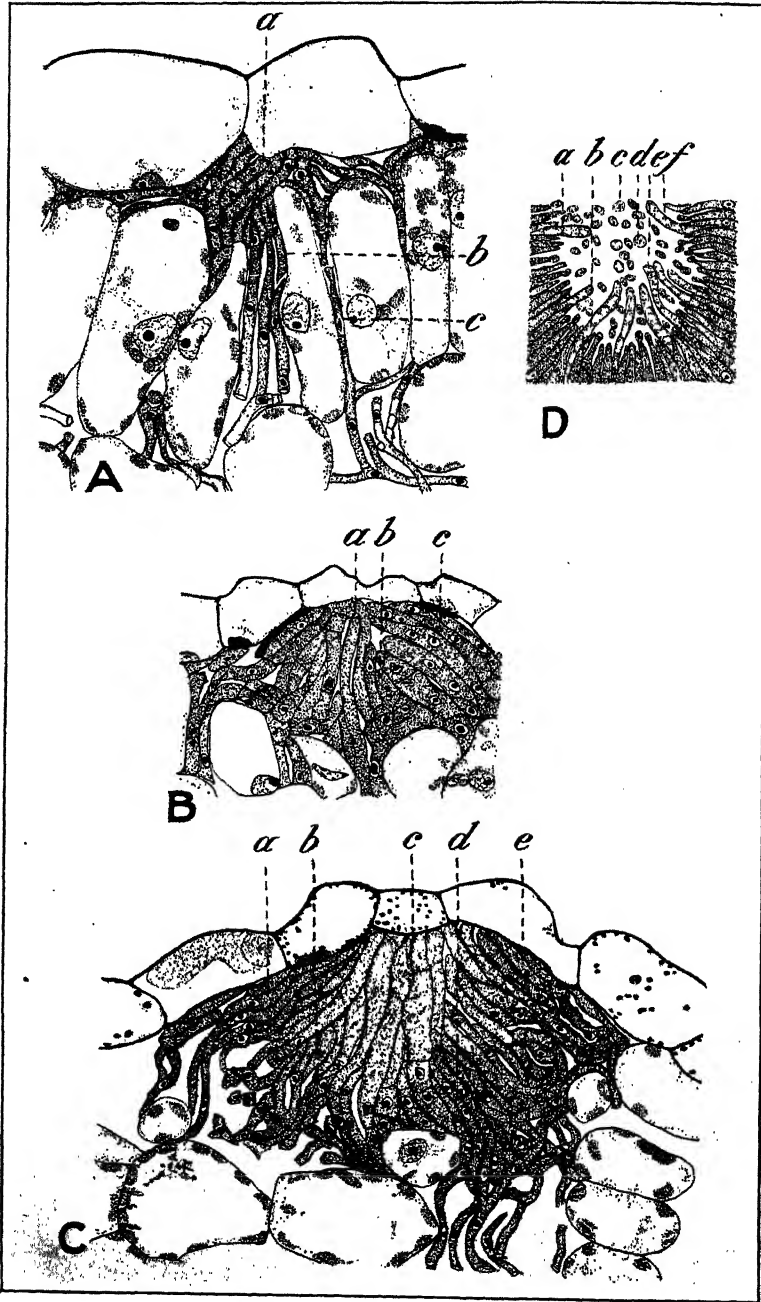
An early stage of pycnial development is represented in Plate 4, A. Hyphae with dense contents (pl. 4, A, *b*, *c*) force a passageway up between palisade cells and between the epidermal and palisade layers. These hyphae branch freely, forming a subepidermal mat in which at first no organization is evident. Soon, however, they begin to converge toward a central point, or if the area is unusually large there may be two centers of development. In Plate 4, A, this convergence is just beginning. Hyphae are turning toward *a*. In Plate 4, B, a slightly later stage drawn to the same scale, the centering

EXPLANATORY LEGEND FOR PLATE 3

A.—(Agr.) Median section of 4-day infection. Disintegrating primary hypha, *d*, *f*. Dying intracellular hypha, *c*. Living intracellular hypha at *a* passes into intercellular space at *b*. Intracellular hypha at *h*. Intercellular mycelium at *e*, *g*, *j*, *k*. Haustorium at *i*. $\times 730$.

B.—(Agr.) Three-day infection. Two primary hyphae, *a*, *b*, in one epidermal cell. Intercellular hyphae at *d*. Hypha on outer surface of host at *c*. $\times 1,130$.





is more evident. By proliferation the few original hyphae have multiplied, and from all sides they are aimed at the common point, *a*. The cells are regularly uninucleate. A few (pl. 4, B, *b*, *c*) contain two nuclei, but these are the rapidly growing terminal cells of hyphae, and nuclear division has not yet been followed by cell division.

A later stage (from 5-day material) is represented in Plate 4, C. This subepidermal mass now has the form of a thick biconvex lens. The raised epidermis, out of its normal food relations with the mesophyll tissue and stretched by the growing fungous mass beneath it, is somewhat flattened and is dying. The hyphae have multiplied by further branching and are well centered. The uppermost hyphae, *a*, *e*, running just beneath the epidermis and parallel to it, have remained slender and are dense in content. These will later give rise to paraphyses. The hyphae at the center beneath *c* are upright and columnar, and their great size and turgor and their vacuolated cytoplasm suggest that they are exerting force in lifting the epidermis and are serving as buffers against its pressure. Groups of smaller hyphae (pl. 4, C, *b*, *d*) are forcing their way inward from the margin. These are the first of the pycniosporophores which will later produce the spores.

The number of pycniosporophores now increases rapidly. Each arises from a marginal hypha and grows inward. These soon form a continuous compact marginal layer, and the whole pycnium arches out as new pycniosporophores are interpolated between the older. It soon forms a rounded mass, displacing mesophyll cells as it grows. This expansion of the pycnium releases the buffer cells from their pressure against the epidermis. In Plate 6, B, is drawn at high magnification the central group of buffer cells, *b*, and the inner wall of an epidermal cell, *a*. The buffer cells are no longer pressed tightly against one another and against the epidermis. They are somewhat lax and their tips lie free in the center of the pycnium. As before, their cytoplasm is vacuolate and the nucleus of each is far back from the tip. Pycniosporophores are encroaching upon their space at *c* and *d*.

In Plate 5, A, is a somewhat later stage. The tips of the growing paraphyses have met at the apex and turned outward, exerting pressure against the epidermis at *a*, where they will soon break through. It is to be noted that there is a single nucleus at the growing tip of each paraphysis. Surrounding the main body of the pycnium are one or two layers of short cells forming a more or less definite pycnial wall. Numerous pycniosporophores extend inward and upward from this wall, curving toward the future ostiole. The buffer cells, *b*, in this preparation are present but are stained too faintly to be drawn in detail.

By the sixth day, under favorable conditions, the first pycnia have matured and opened and are shedding spores through the ostiole or apical opening. Plate 5, B, represents a newly opened pycnium. It has expanded farther, making space for itself by crushing adjoining

EXPLANATORY LEGEND FOR PLATE 4

A.—(Trit.) Four-day infection. Hyphae forcing passageway up between palisade cells at *b*, *c*, to start a pycnium at *a*. $\times 730$.

B.—(Hor.) Slightly older pycnium in 6-day infection. Hyphae converging at *a*. Young pycniosporophore at *b*. Young paraphysis at *c*. $\times 730$.

C.—(Trit.) Larger pycnium from 5-day infection. Buffer cells at *c*, young pycniosporophores at *b*, *d*, and paraphyses at *a* and *e*. $\times 730$.

D.—(Hor.) Center of mature pycnium from 9-day infection. Buffer cells at *a*, *b*, *c*, *d*, *e*, *f*. $\times 730$.

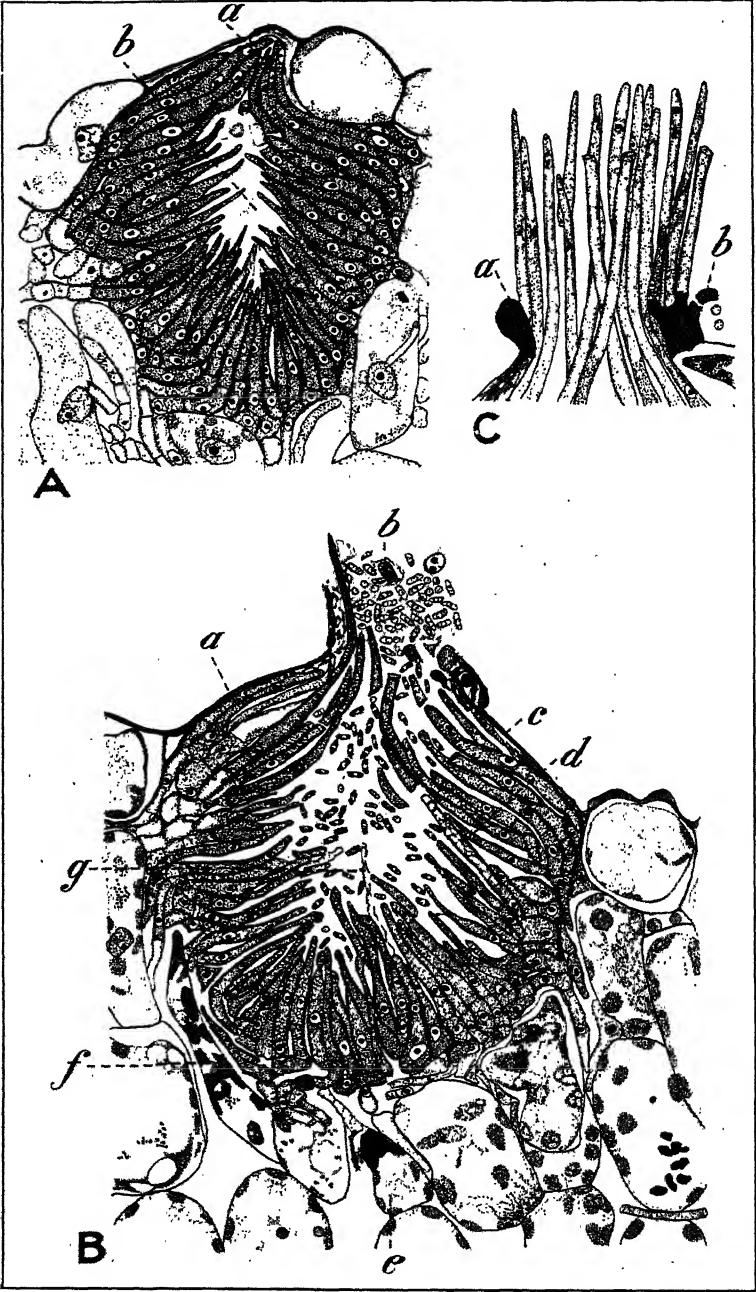
chlorophyll cells, *e*, *f*, and by lifting and flattening the epidermis at the sides, *a*, *c*, and rupturing it at the top, *b*. From its outer wall or cortex the pycniosporophores extend inward, and at their slender tips pycniospores are formed and set free into the central cavity.

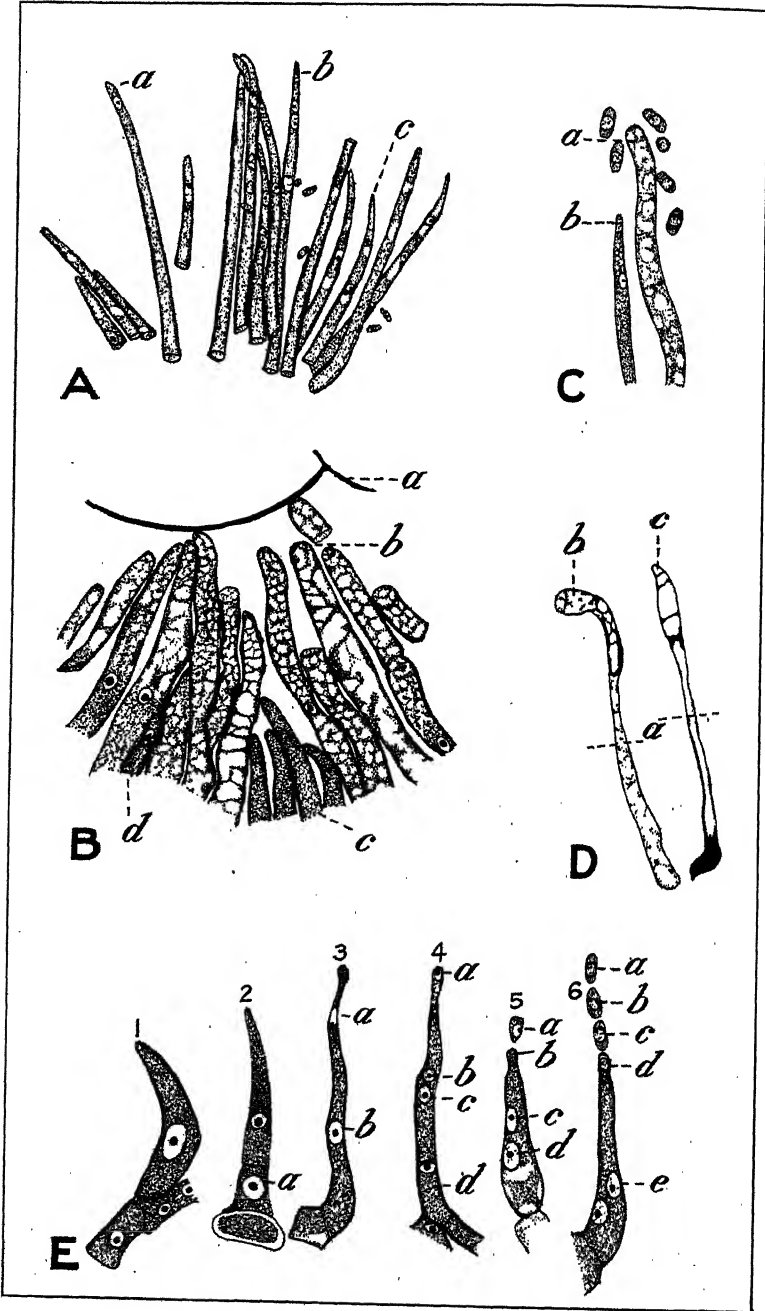
Details of the development of pycniosporophores and spores are presented in Plate 6, E. The young pycniosporophores found in the pycnium before it opens (pl. 6, E, 1) is thick and often curved, and tapers to a rather blunt tip. It contains dense cytoplasm and a single nucleus. As it grows (pl. 6, E, 2) the nucleus divides and sometimes, but not always, a septum near the base gives rise to a short stalk cell (2, *a*, and 4, *d*). The terminal cell continues to grow in length. Pycniosporophores are crowded together, and details of shape, length, and thickness vary with the available space. In 3 the primary nucleus has divided. One of the two daughter nuclei (3, *b*) has remained near the base and is growing rapidly; the other (3, *a*) has not grown and is migrating toward the swelling tip of the cell. A little later this nucleus reaches the tip (4, *a*), and the basal nucleus divides again (4, *b*, *c*). In Plate 6, E, 5, the spore, *a*, has been abscised, the tip of the cell, *b*, is reshaping to form a second spore, and another nuclear division has provided the extra nucleus, 5, *c*, which has begun to move toward the tip. This process continues until, as in Plate 6, E, 6, there is a row of free spores, *a*, *b*, *c*, beyond the tip, a fourth forming at *d*, and provision for a fifth in the nucleus, *e*. This procedure keeps on indefinitely. The first spores emerge through the ostiole soon after the opening of the pycnium (pl. 5, B, *b*), doubtless carried out in the swelling slime in which they are embedded.

The buffer cells, which filled the central area in the younger pycnia (pl. 4, C, *c*, and pl. 5, A, *b*), are now inconspicuous, vacuolated cells, extending somewhat farther into the central cavity than the pycniosporophores. Plate 4, D, represents the central cavity of a newly opened pycnium. The buffer cells (seen in side view in pl. 4, D, *a*, *b*, *e*, and in cross-section at *c* and *d*) no longer form a compact group; they have been separated by the pycniosporophores that squeezed in between them. They are still, however, long, coarse, blunt-tipped cells with vacuolated cytoplasm. In Plate 6, C and D, are drawn separately at high magnification buffer cells in different stages of deterioration. In C is drawn a buffer cell, *a*, with a pycniosporophore, *b*, alongside. The buffer cell is longer, coarser, and has a blunter tip and thinner content. D, *b*, represents a dying, and D, *c*, a dead buffer cell. The dotted line, *a*, in each is at the level of the tips of the pycniosporophores. At *g*, in Plate 5, B, is a withered buffer cell, and at *d* is one that is still turgid. The time at which the buffer cells disappear varies greatly. A few are still present in pycnia of 20-day infections. It would appear, then, that the buffer cells serve only the transient purpose of helping to lift the epidermis above the young growing pycnium. So far as noted they produce no spores, although they may be present long after spore production has begun.

EXPLANATORY LEGEND FOR PLATE 5

- A.—(Agr.) Nearly mature pycnium from 7-day infection. Paraphyses turned outward at *a* to break epidermis. Numerous pycniosporophores. Buffer cells at *b*. $\times 730$.
 B.—(Trit.) Newly opened pycnium in 6-day infection. Paraphyses at *a*. Crushed epidermis at *c*. Buffer cells at *d* and *g*. Spores emerging at *b*. Crushed mesophyll cell at *e* and *f*. $\times 730$.
 C.—(Trit.) Six-day infection. Turgid young paraphyses between broken epidermal cells at *a*, *b*. $\times 730$.





The paraphyses, which in an earlier stage (pl. 5, A, *a*) were about to rupture the epidermis, have now (pl. 5, B, *b*) broken through, forming the ostiole. They soon elongate into a brush of stiff-looking hyphae, each tapering to a point (pl. 5, C; 6, A) containing living cytoplasm and 1, 2, or even 3 nuclei.

Pycnia grow considerably after the first maturity, and older pycnia that have produced spores for a week or more sometimes open wider. In extreme cases the ostiole nearly equals in size the cross diameter of the pycnium.

The number of spores produced is enormous. The pycnia of an infection maintain a good-sized drop of "pycnial exudate," a golden brown, sirupy liquid filled with spores, on the upper surface of the leaf throughout their active period. When two or more infections are close together the drops often coalesce into one still larger drop.

HETEROTHALLISM

The course of development of all infections of *Puccinia graminis* studied is essentially similar up to the time when the pycnia mature. From this point on, their progress diverges into one or other of two channels leading to very different ends. Some of the infections produce aeciospores and others remain sterile.

If *Puccinia graminis* is heterothallic and there are only two sexual groups, each plant of the gametophyte generation is either (+) or (-). Neither a (+) nor a (-) strain when isolated can form diploid mycelium or aeciospores. Only when (+) and (-) are brought together can fertile aecia form.

A count was made of all the infections on a plant 32 days after inoculation. This is long after the first appearance of open aecia. The plant was left untouched during the growth of the infections. The infections were classified as (1) singles (i. e., isolated infections) without open aecia, (2) singles with aecia, (3) doubles (i. e., two infections in contact) without open aecia, and (4) doubles with aecia. The count showed 37 singles without open aecia, 2 singles with open aecia, 14 doubles without and 15 doubles with open aecia. Theoretically, the singles and 50 per cent of the doubles should remain sterile, and the other 50 per cent of the doubles should produce aeciospores. The above count fits the expectation closely—only the two fertile singles are out of line. Counts of other infected plants have been made. Some of them did not come so close to the theoretical expectation, especially when infections were somewhat crowded. In these cases there were more infections with aecia than would be expected.

A few days later (36 days after inoculation) the infections on the plant described above were fixed. Sections through typical sterile

EXPLANATORY LEGEND FOR PLATE 6

- A.—(Trit.) Tuft of young paraphyses from pycnium of 6-day infection. A paraphysis may contain 1 (a), 2 (c), or 3 (b) nuclei. $\times 730$.
 B.—(Trit.) Eight-day infection. Group of buffer cells from center of half-grown pycnium. Epidermal wall at *a*. Buffer cells at *b*. Young pycniosporophores at *c*, *d*. $\times 1,460$.
 C.—(Trit.) Eight-day infection. Buffer cell, *a*, and pycniosporophores, *b*, from open pycnium. $\times 1,460$.
 D.—(Trit.) Eight-day infection. Dying buffer cell, *b*, and dead buffer cell, *c*, from mature pycnium. Level of tips of pycniosporophores at *a*. $\times 1,460$.
 E.—(Trit.) Details showing development of pycniosporophores and spores. 1. Young pycniosporophores. 2. Older, with stalk cell at *a*. 3. Nucleus divided. One daughter nucleus, *b*, at base; the other, *a*, moving toward tip. 4. Nucleus, *a*, at tip where spore is forming; basal nucleus, divided, *b*, *c*. 5. Spore freed at *a*; tip of pycniosporophore reshaping to form second spore; two nuclei below, *c*, *d*. 6. Three spores, *a*, *b*, *c*, freed, a fourth forming at *d*, and an extra nucleus at *e*. $\times 1,460$.

and fertile infections from this plant are represented semidiagrammatically in Plate 7, A and B. Plate 7, A, is a median section through an isolated infection. The infected area of the leaf is greatly hypertrophied, as can be seen by comparing the normal thickness of the leaf at *e* with its thickness in the infection. In spite of the great age of the rust, pycnia are numerous and still active. There was an abundant pycnial exudate on the living leaf, some of which adhered through the processes of fixing, washing, etc., and is still present. (Pl. 7, A, *a, c, d*.)

In addition to the pycnia, other fungous structures have formed near the lower surface of the leaf. (Pl. 7, A, *f, g, h, i, j, k, l*.) These are nearly spherical and consist of an outer shell of small-celled pseudoparenchyma and a central mass of larger rounded cells. They are dead or nearly dead. A cytological examination of many such isolated infections shows that these structures are of regular occurrence. There are from 3 or 4 up to 2 dozen or more in each infection. They are sterile aecia.

Plate 7, B, is a section through two adjoining infections separated only by a vein at *c*. Here, too, the leaf tissue is hypertrophied. Pycnia are present (pl. 7, B, *a, b, d, e, f*), but they have ceased to function, and the remaining bits of exudate are dried and dead. Here, too, a row of fungous structures has formed in the leaf near the lower surface (pl. 7, B, *g, h, i, j, k, l*), but with one exception (*i*) they have developed into normal aecia and are shedding spores freely. The vein between these two infections is impenetrable to mycelium, but the pycnial exudate of the two may easily have been mixed.

HISTORY OF ISOLATED INFECTIONS

Material was available for a study of the processes leading to the sterile condition shown in Plate 7, A.

The newly opened pycnium and the young paraphyses have been described. Within and around the pycnium, cells are uninucleate. (Pl. 5, B.) A young paraphysis about to break through the epidermis contains a single nucleus near its apex. (Pl. 5, A, *a*.) Soon after its emergence the nucleus may divide. Some of the paraphyses in the tuft in Plate 5, C, contain more than one nucleus. In the brush of paraphyses in Plate 6, A, a single cell may contain 1 (pl. 6, A, *a*), 2 (*c*), or even 3 (*b*) nuclei. So far as noted, the emergent part of the paraphysis is nonseptate. Both of these tufts occur in 6-day infections. The pycnia have been open only a few hours.

The paraphyses on the pycnia of older sterile infections show the same nuclear content. In Plate 7, C, drawn from a pycnium in a 14-day infection, the paraphyses and their nuclei are somewhat larger, but they still contain two or, less commonly, three nuclei.

In Plate 7, D, is drawn the edge of a pycnium, *a*, and mycelium adjacent to it, *b, c*, from a 10-day infection. The pycniospores of the infections on this leaf were mixed a few hours before the material

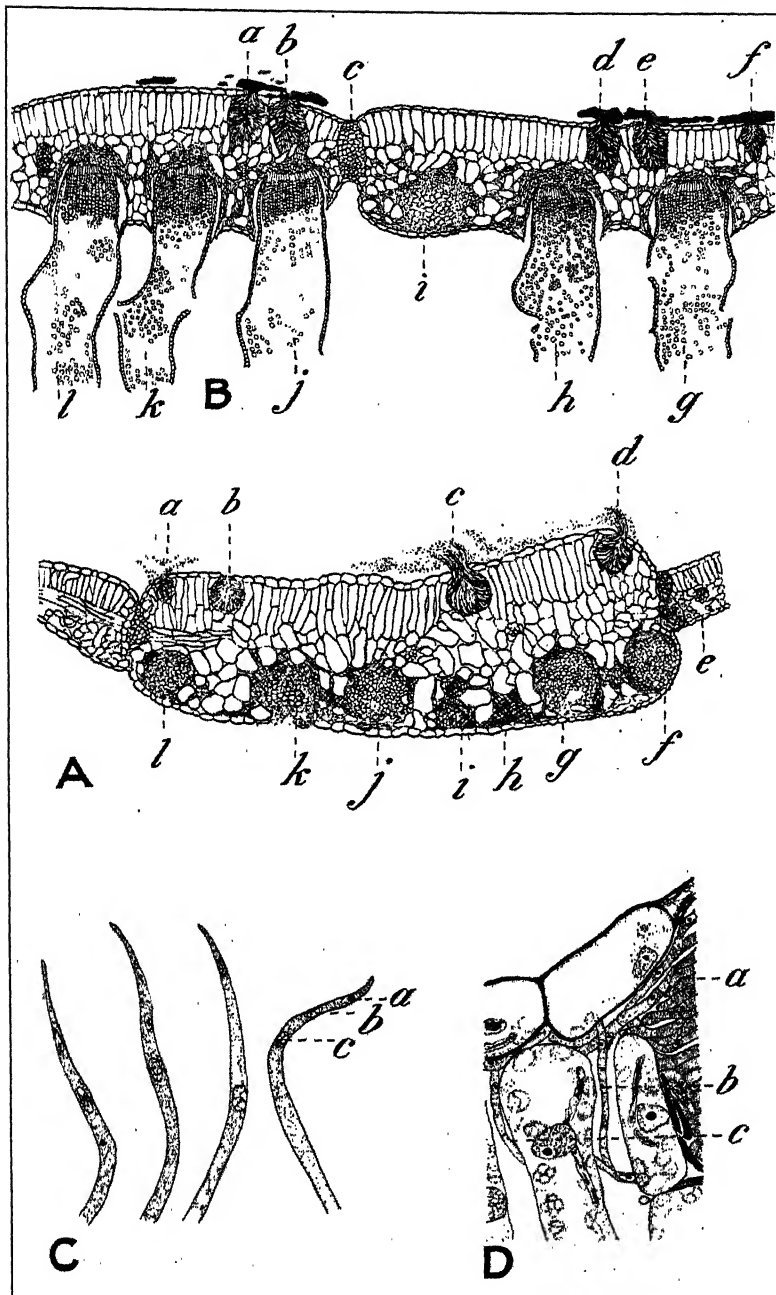
EXPLANATORY LEGEND FOR PLATE 7

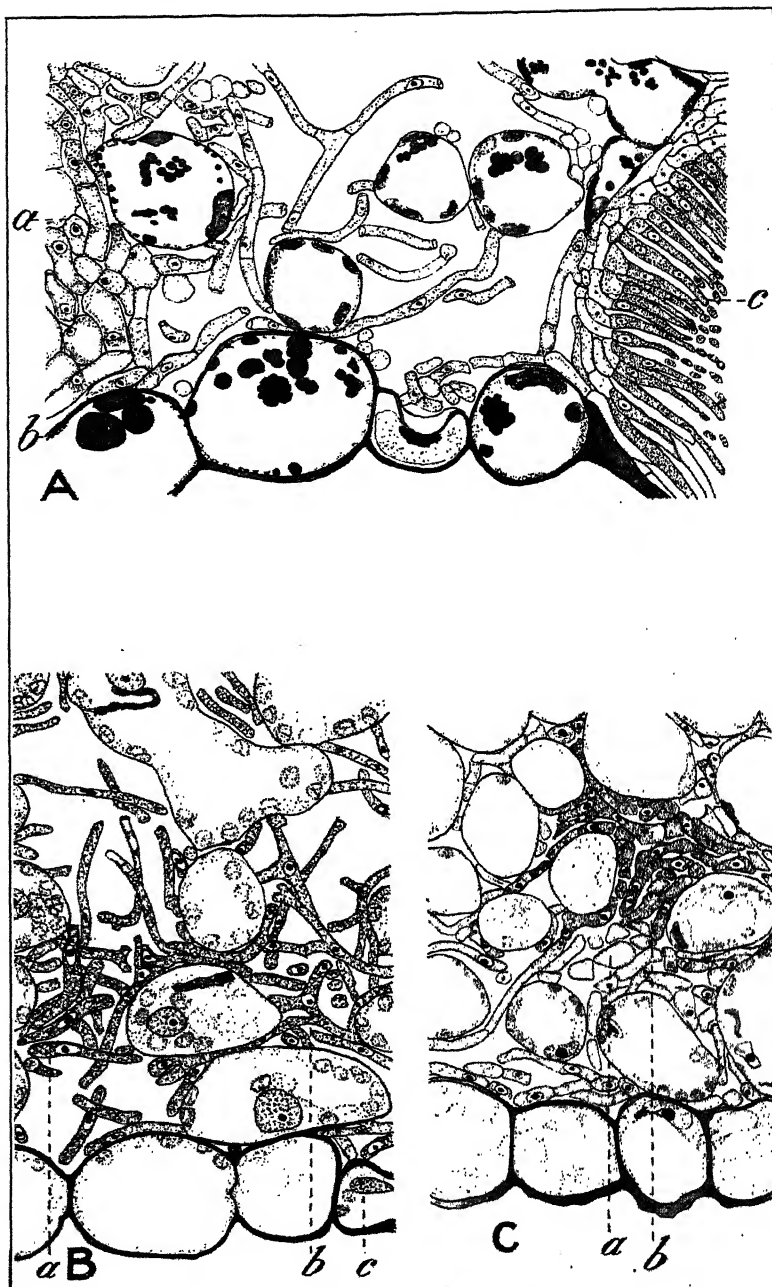
A.—(Agr.) Semidiagrammatic drawing of 36-day sterile infection. Living active pycnia at *a, b, c, d*. Sterile dead aecia at *f, g, h, i, j, k, l*. Healthy leaf tissue at *e*. $\times 40$.

B.—(Agr.) Diagram of two fertile infections separated by a vein at *c*. Dead pycnia at *a, b, d, e, f*. Fertile aecia at *g, h, j, k, l*. Sterile aecium at *i*. $\times 40$.

C.—(Agr.) Paraphyses from pycnium of 14-day sterile infection. Paraphysis contains two or sometimes three nuclei (*a, b, c*). $\times 730$.

D.—(Agr.) Margin of a pycnium, *a*, and adjacent mycelium, *b, c*, from a 10-day sterile infection. $\times 640$.





FOR EXPLANATORY LEGEND SEE PAGE 597

was fixed. One or two of the other pycnia of this infection give evidence of it, but in this pycnium and the mycelium around it all fungous cells are uninucleate, so far as noted.

In Plate 8, A, from a 9-day sterile infection, is drawn the edge of a pycnium, *c*, the edge of a young aecium, *a*, and the area between. Pycnia commonly form beneath the upper surface of the leaf, but this one, as is not rare, opens downward. In the pycnium, the aecium, and the mycelium between the two, the cells are uniformly uninucleate.

The earliest indication of aecia in sterile infections is the formation of denser wefts of mycelium in the large air spaces of the mesophyll tissue near the lower surface of the leaf. Plate 8, B, from a 10-day infection, depicts such a one. The slender hyphae, *a*, *b*, run in all directions between the host cells, branching freely. They are composed of uninucleate cells.

The hyphae in this loose tangle grow and branch rapidly and become interwoven into a solid little mass of pseudoparenchymatous tissue. (Pl. 8, C.) This is early differentiated into an outer half of larger cells with thin contents (pl. 8, C, *a*) and an inner half of smaller cells with dense contents (pl. 8, C, *b*).

The differentiation into areas becomes accentuated with further growth. Plate 9, A, from a 10-day sterile infection, represents semi-diagrammatically, at low magnification, a larger sterile aecium, with its lower region, *c*, of large rounded vacuolate cells, its upper region, *b*, of small dense angular cells, and an outer covering, *a*, surrounding the whole, consisting of several layers of small cells. The fungous mass has crushed or pushed aside mesophyll cells as it expanded. The central region between *b* and *c* is drawn enlarged (same scale as preceding drawings) as Plate 9, B. With one doubtful exception (pl. 9, B, *b*), the cells are uninucleate throughout. The central area between *a* and *c* is composed of cells very rich in cytoplasm, but there is no indication of activity leading to spore formation.

The time of formation of these sterile aecia varies. Rarely, aecia may begin to form before the opening of the first pycnium, but more commonly about the ninth or tenth day. As the mycelium spreads radially, new aecia arise in succession out toward the margin.

In Plate 9, C, is drawn at lower magnification a small specimen of a later stage. The cells are still living but impoverished, the outer and inner regions alike consisting now of nearly empty cells.

Plate 10, A, shows a sterile aecium from a 36-day infection, the age shown in the diagram in Plate 7, A. The whole fungous mass is dead. In the center are greatly enlarged rounded cells, empty and clear. Sometimes a break in this mass leaves an irregular clear area (pl. 10, A, *c*) which may, as in this case, be threaded by fine hyphae, now also dead. Around the outside (pl. 10, A, *a*) is a denser shell consisting of several layers of smaller closely packed hyphae. There were 29 such sterile aecia in the infection from which this one is drawn.

EXPLANATORY LEGEND FOR PLATE 8

A.—(Trit.) Part of a pycnium, *c*, and a young aecium, *a*, and the mycelium between, from a 9-day sterile infection. Lower epidermis at *b*. $\times 730$.

B.—(Agr.) Very young aecium composed of slender hyphae with uninucleate cells, *a*, *b*, from 10-day sterile infection. Lower epidermis at *c*. $\times 730$.

C.—(Hor.) Slightly older aecium from a 9-day sterile infection in a petiole. Differentiated into an inner half (*b*) of small dense cells and an outer (*a*) of larger vacuolate cells. All uninucleate. $\times 730$.

Pycnia in sterile infections continue spore production for a surprisingly long period. In Plate 10, C, is drawn (at low magnification and in outline only) an active pycnium from an infection 51 days old. There are fresh spores in the central cavity and outside. It has undergone considerable modification, however. The pycnium as a whole has enlarged, its outer wall has thickened (pl. 10, C, *b*, *c*), its primary paraphyses (pl. 10, C, *a*) have withered and died, and secondary paraphyses have grown out from a lower level in the pycnial wall. In rare cases, paraphyses may arise from the floor of the pycnium as well as from its side walls. This is accompanied by a widening of the ostiole, until it sometimes equals in diameter the cross diameter of the whole pycnium. The majority of the pycnia in infections over 3 weeks old show these secondary changes in varying degrees.

In Plate 9, D, from a 51-day infection, is drawn part of a group of secondary paraphyses. They are long, slender cells, still living, although the nuclear content is becoming vague. They are predominantly binucleate, so far as can be determined. At one side (pl. 9, D, *a*) are dead remnants of the earlier paraphyses. Plate 9, D, is drawn at the same magnification as the full-grown primary paraphyses in Plate 7, C. A comparison of the two shows that the secondary paraphyses are decidedly longer—almost twice as long—as the primary.

In one lot of material, derived from teliospores on *Hordeum jubatum*, the pycnia of 20-day infections frequently show a different type of secondary change. Here the pycnium stops making spores, and the pycniosporophores elongate, growing to the ostiole and even extending through it, or else becoming snarled up in the central cavity. Meanwhile, at the base of the pycnium there is an irregular type of renewed growth, and there form one or more dense masses of hyphae extending into the deeper tissues of the leaf. Plate 10, B, shows an extreme case of this degenerative secondary growth. At *a* are the withered primary paraphyses. Plate 10, B, *b*, marks the level of the original base of the pycnium, and *c* the deeper secondary growth. The pycnium is hopelessly disorganized and the mass would hardly be recognized as pycnial in origin if intermediate gradations between it and the normal pycnium were not available.

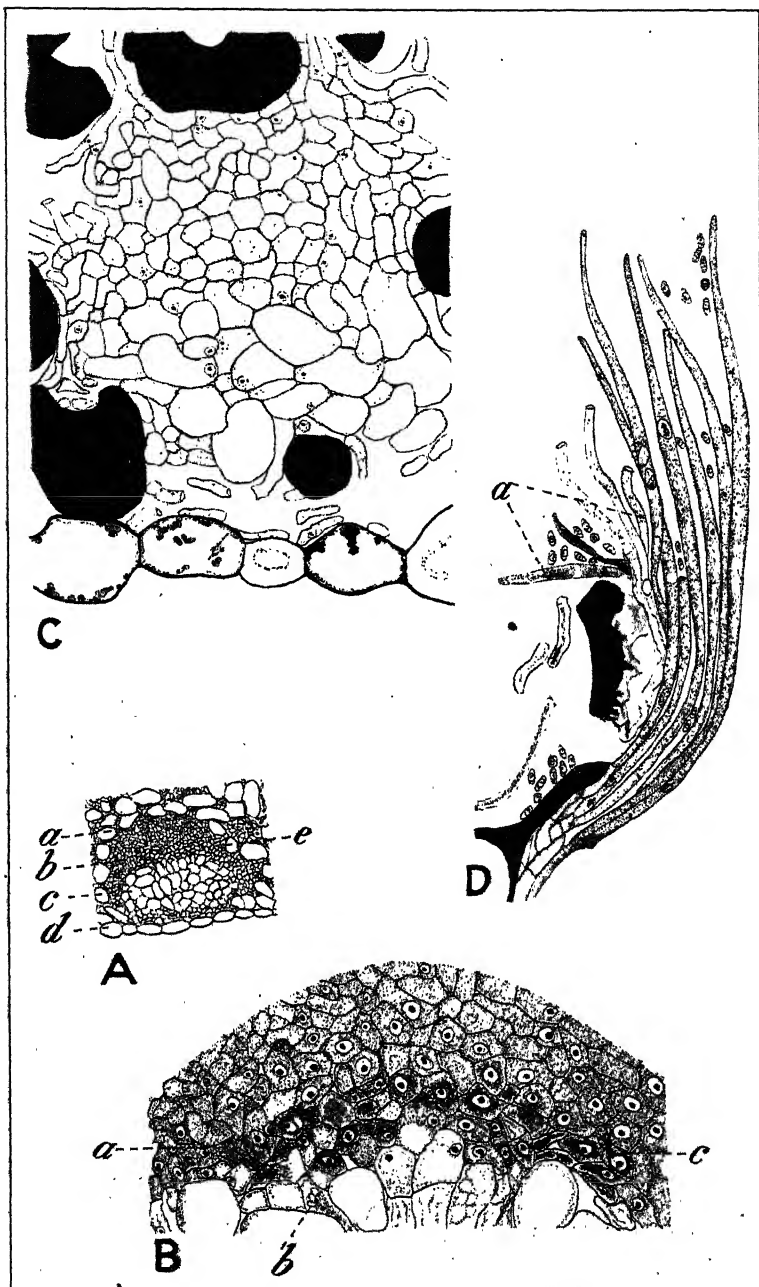
DEVELOPMENT OF FERTILE INFECTIONS

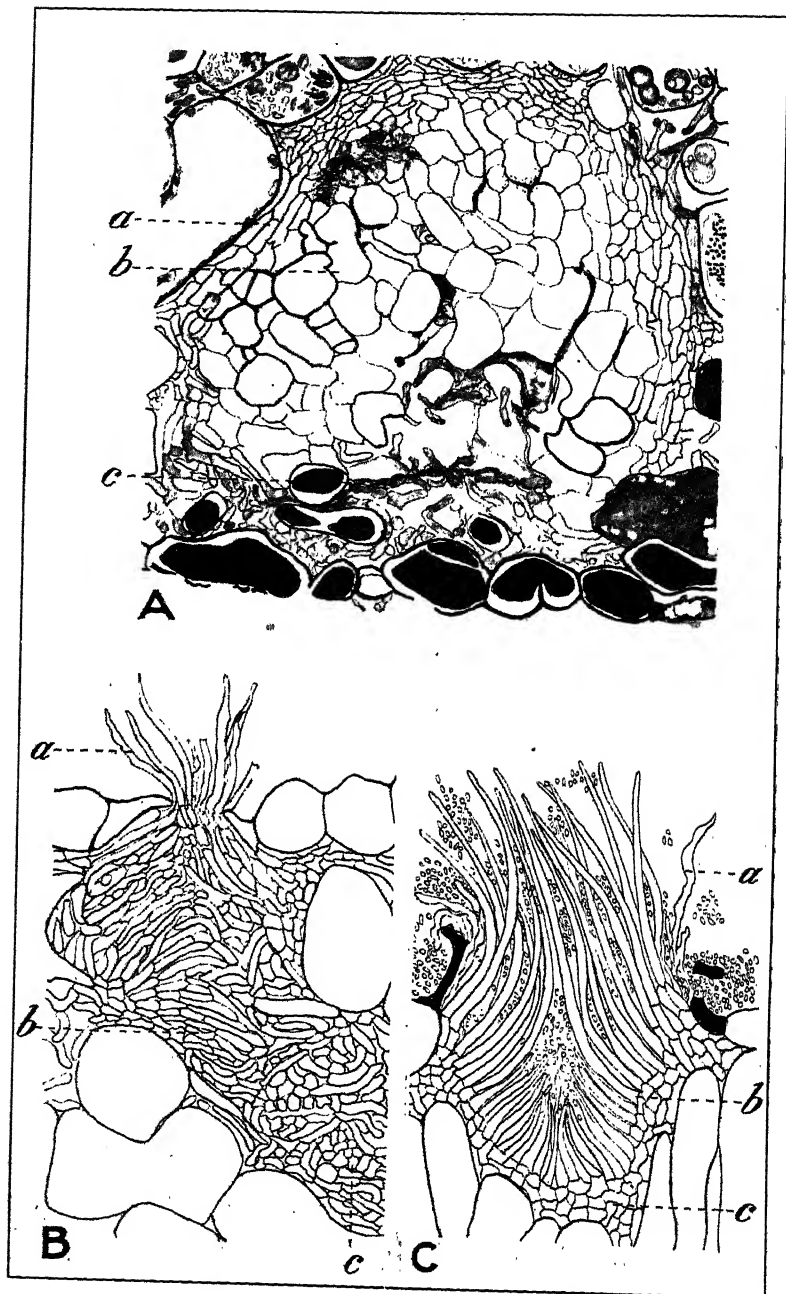
The course of development leading to normal fertile aecia also has been traced.

In Plate 11, C (corresponding to Plate 7, D, of the unfertilized series), is drawn from a 10-day infection the edge of a pycnium, *h*, and the tissues adjoining it. Pycniospores in this case were not mixed by hand, but another infection was so close to this that the pycnial exudate of the two probably was continuous. Above the leaf epidermis (pl. 11, C, *b*) are pycniospores, *a*. Just beneath the epider-

EXPLANATORY LEGEND FOR PLATE 9

- A.—(Trit.) Semidiagrammatic drawing of larger aecium from a 10-day sterile infection. Feeding tissue at *a*. Small dense cells from *b* to *c*. Large nearly empty cells at *c*. Lower epidermis at *d*. $\times 115$.
 B.—(Trit.) Region between *b* and *c* of A enlarged. Cells denser from *a* to *c*, but all uninucleate. Doubtful binucleate cell at *b*. $\times 730$.
 C.—(Trit.) Small sterile aecium from a 14-day infection. Cells impoverished. $\times 640$.
 D.—(Hor.) Tuft of secondary paraphyses from a living active pycnium of a 51-day sterile infection. Primary paraphyses now dead at *a*. $\times 730$.





FOR EXPLANATORY LEGEND SEE PAGE 599

mis at *g* are the bases of paraphyses, and adjoining these in the wall of the pycnium are fungous cells, *c*, *d*, containing two or three nuclei. Growing downward between palisade cells from this region are slender hyphae (pl. 11, C, *e*, *f*, and in the next section, pl. 11, D, *a*, *b*, *c*), the cells of which contain two or three nuclei.

The fact that diploid hyphae may take their origin at the base of the paraphyses suggests at once that the diplophase may arise through the agency of paraphyses. It is at least hypothetically possible that a pycniospore from another pycnium might be brought in contact with a paraphysis, enter, its nucleus pass down to the base, and there in association with one from the same pycnium start a binucleate hypha; or that a (+) and a (−) pycniospore should become closely associated, the pair of nuclei enter the paraphysis, and move down to the base.

Attempts to determine whether this happens meet with difficulties. As already noted, the native nuclear content of the paraphysis is not uniform. There may be 1, 2, or even 3 nuclei in the extruded part of the young paraphysis before the introduction of spores from other pycnia. (Pl. 5, C; pl. 6, A.) In older isolated infections also the number may be 1, 2, or 3. (Pl. 7, C; pl. 9, D.)

Moreover, pycniospores transferred from another pycnium are indistinguishable from those grown there, and when a dozen spores are seen adhering to a paraphysis it can not be told whether they are native or introduced. The spores are so minute that the actual entry of a spore nucleus, if it takes place, would be difficult to detect.

One lot of material (14-day infection) was fixed a few minutes after transferring pycniospores to it. In several instances paraphyses of the type shown in Plate 12, D, were found in this lot. An occasional paraphysis might contain one or two nuclei (pl. 12, D, *c*), but alongside it would be others containing one or two larger nuclei, *a*, and a group of similarly staining small granules, *b*, which may or may not be nuclei.

In Plate 11, B, is drawn another group of paraphyses from the same lot of material as in Plate 12, D. Here, too, the evidence is inconclusive, but suggestive. In addition to the regular nuclei there are small granules, each surrounded by a narrow clear space, which may or may not be nuclei. A total of four (pl. 11, B, *a*) and even five (B, *b*) is not rare.

Whether or not the paraphyses serve in this capacity, they are probably not the only means by which binucleate hyphae are initiated. In Plate 12, A (from the same infection in which the materials for Plate 11, C and D, were found), is drawn the area from a pycnium, *a*, *e*, on the one side, to the margin of a very young aecium, *h*, on the other. In the pycnial wall the cells are partly drained and the nuclei indistinct. Those that can be determined are uninucleate. At the inner end of the pycnium, however, are several binucleate cells. (Pl. 12, A, *a*, *b*, *c*.) Other similar cases have been seen.

EXPLANATORY LEGEND FOR PLATE 10

A.—(Agr.) Dead haploid aecium from a sterile 36-day infection. Outer cortex of small cells at *a*; larger central cells at *b*; finer dead hyphae at *c*. × 333.

B.—(Hor.) Teratological growth of a pycnium in a 20-day sterile infection. Dead paraphyses at *a*. Original base of pycnium at *b*. Deeper abnormal growth at *c*. × 320.

C.—(Hor.) Semidiagrammatic drawing of active pycnium from 51-day sterile infection. Dead primary paraphyses at *a*, supplanted by secondary paraphyses. Pycnial wall thickened at *b*, *c*. × 320.

How pycniospores brought to the surface of the leaf outside of the pycnium could give rise to binucleate cells at the base of the pycnium is not known with certainty. The evidence at present is limited to a single slide. In this preparation there were two open pycnia extruding spores and there was a continuous mass of exudate on the leaf from one pycnium to the other.

In Plate 11, A, *a*, are represented at high magnification three of the pycniospores within one of the pycnia. In Plate 11, A, *b*, is a similar spore in the exudate outside of the pycnium. In *c* is a spore in the exudate which is pushing out at one end. In *d* the spore has pushed out a short tube at both ends. The spores in *e* and *f* are larger and growing at both ends. In *g* two germinating pycniospores have germ tubes almost meeting at the tips, and *h* shows a similar pair. In *i* and *j* the shape suggests a fusion of two germinating spores, although, of course, other interpretations are possible. Both *i* and *j* occur in the mixed exudate midway between the two pycnia. In *k*, *l*, and *m* are later stages, in which the broadening hyphae are growing toward one or the other of the ostioles.

Some accident before fixation had broken the paraphyses off from one of the two pycnia, leaving the open ostiole with spores issuing from it. In Plate 11, E, is represented the ostiole, *b*, filled with spores, a few of the pycniosporophores, *d*, a bit of the leaf epidermis, *a* to *e*, two broken remnants of paraphyses, *c* and *f*, and several hyphae, *g*, *h*, and *i*, which resemble the younger germinating spores. One of them here, *h*, and one or two others in the next section can be followed directly to the ostiole at *a*. It has not been possible to trace them down into the pycnium.

On this basis, pycniospores can germinate, forming delicate germ tubes, then fuse by pairs, and the fusion cell form a larger, heavier hypha which grows in the liquid of the pycnial exudate to the ostiole. Why, if this can take place, it has not been seen frequently, is not clear. All of this material was fixed by daylight between 8 a. m. and 8 p. m. Perhaps pycniospores grow at night.

Between the pycnium and the edge of the aecium (pl. 12, A) the mycelium is made up of a mixture of gametophytic and sporophytic hyphae. The hyphae run irregularly. No one binucleate hypha can be traced all the way from pycnium to aecium, but several scattered binucleate cells are present. (Pl. 12, A, *c*, *d*, *f*, *g*.) There are no diploid cells at *h*, but two or three sections farther on, at the center of the young aecium, there are several.

The aecium begins in the lower mesophyll tissue of the leaf as a loosely woven snarl of slender intercellular hyphae. In Plate 12, B, is drawn a section through a young aecium from a 13-day infection. It is the youngest of a series formed in quick succession and is located near the margin of the infection. Only the presence of an occasional

EXPLANATORY LEGEND FOR PLATE 11

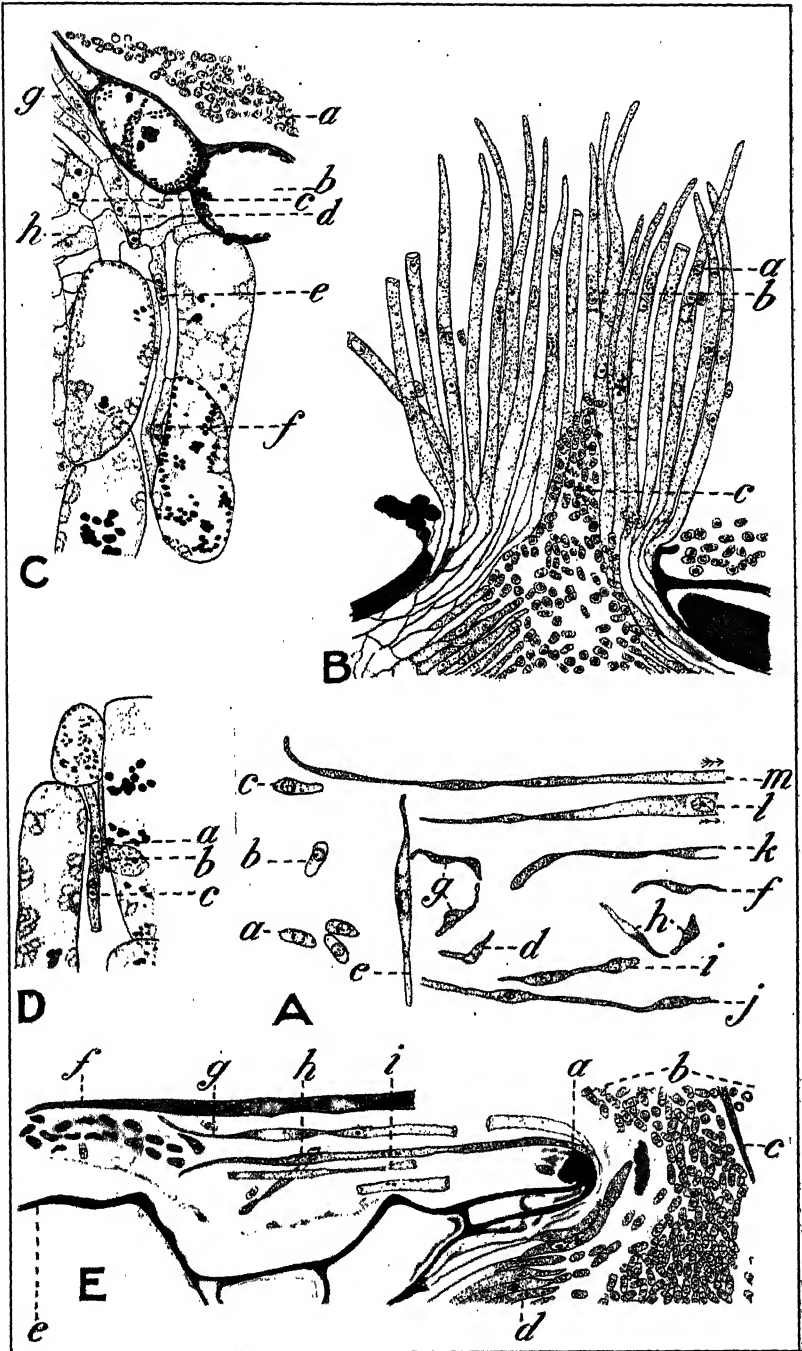
A.—(Trit.) Drawn from a mass of exudate between two pycnia in 6-day material. In *a* and *b*, ungerminated spores. Stages of germination in *c*, *d*, *e*, and *f*. In *g* and *h* are pairs of germinating pycniosporophores. In *i* and *j* two germinating pycniosporophores may have fused. Later stages of growth in *k*, *l*, and *m*. $\times 1,400$.

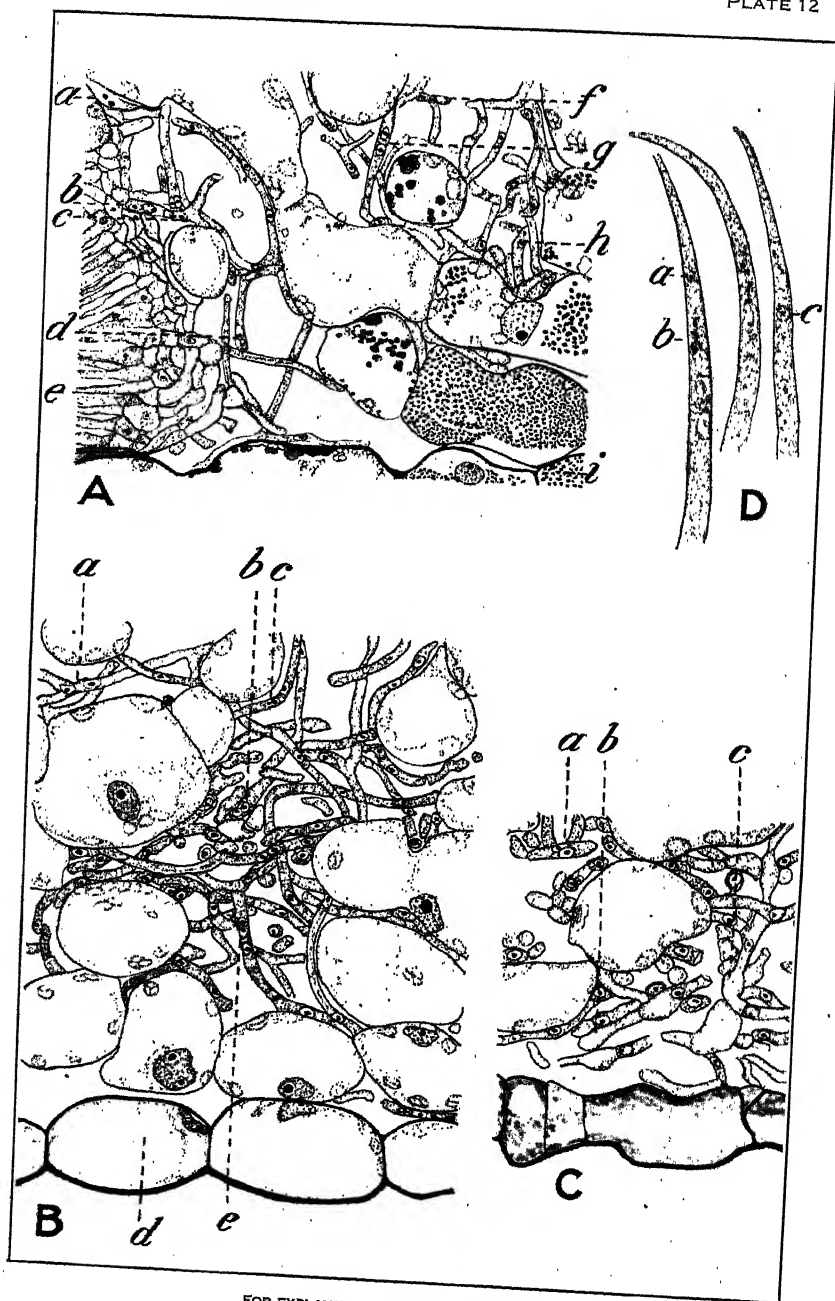
B.—(Trit.) Same infection as A. Tuft of paraphyses showing nuclear content, *a*, *b*. Spores at *c*. $\times 730$.

C.—(Agr.) Ten-day fertile infection. Margin of pycnium, *b*, with base of paraphyses at *g*, and spores at *a*, above the epidermis, *b*. Binucleate and trinucleate cells in wall of pycnium, *c*, *d*, and in hyphae from it, *e*, *f*. $\times 640$.

D.—(Agr.) Next section to C. Binucleate cells at *a*, *b*, *c*. $\times 640$.

E.—(Trit.) From same slide as A. Leaf epidermis, *a* to *e*. Ostiole at *b*. Broken paraphyses at *f* and *c*. Hyphae in pycnial exudate growing toward ostiole at *g*, *h*, and *i*. $\times 1,020$.





binucleate cell (pl. 12, B, *a, b, c, e*) distinguishes it from the corresponding stage of development of the sterile aecium. (Pl. 8, B.) There are binucleate cells in the mycelium near by, and older aecia of this same infection show undoubted development toward spore formation.

The next step in development is the appearance of irregular swellings (pl. 12, C), either terminal (*b*) or intercalary (*c*), of the hyphae in the lower half of the aecium. These differ in no wise from those of the corresponding stage in the sterile aecium. (Pl. 8, C.) Here, however, an occasional cell (pl. 12, C, *a*) contains two or more nuclei.

The proportion of diploid cells varies widely in different infections and even in different parts of the same infection. Plate 12, C, and Plate 13, A, are drawn from the same 10-day infection. In Plate 12, C, there is only one binucleate cell. At the base of the pycnium nearest it is a binucleate cell, and there are one or two in the intervening mycelium. In a slightly older aecium of the same infection diploid cells are fairly abundant. (Pl. 13, A, *a, b, c, d, e*.) Moreover, one part of an infection can be quite without diploid cells and can remain permanently sterile while the rest progresses to aeciospore formation. The one sterile aecium amidst fertile aecia in Plate 7, B, at *i*, illustrates this.

In Plate 13, A, already referred to, the hyphae by growth and branching have formed a solid nest, in which the differentiation into a lower half (*d* to *f*) composed of large cells with scant content, and an upper half (*a* to *d*) with smaller denser cells, is well marked.

This small solid knot of hyphae increases rapidly in size. Plate 13, B, shows a narrow strip, at right angles to the surface of the leaf, through the center of the aecium. At *d* is the edge of a hypodermal cell of the leaf. From *c* to *d* are rapidly expanding vacuolate cells which will become the sterile outer half of the aecium. From *b* to *c* are smaller denser cells. From this area spores will arise, and the basal area, *a* to *b*, will serve to transfer food to the spore-bearing region. While sporophytic cells are abundant in the area that is to produce spores (pl. 13, B, *f, g, h*), they are by no means limited to this area. Cells with 2, 3, or even 4 nuclei are scattered from one end of the aecium to the other. (Pl. 13, B, *e, f, g, h, i, j, k, l, m*.)

Infections of this age (13 days) are particularly favorable for a study of nuclei in areas adjoining the aecia. A little later the mycelial cells will be nearly empty and their nuclei will become indistinct. In Plate 13, C, is drawn a corner of an aecium, *e, f*, and mycelium between it and the lower epidermis, *d*. The part of the aecium included is outside of the spore-bearing area, but, as in Plate 13, B, it contains a goodly sprinkling of cells with more than one nucleus (pl. 13, C, *e, f, g*), and in the mycelium outside there is at least an equal proportion

EXPLANATORY LEGEND FOR PLATE 12

A.—(Agr.) Ten-day fertile infection. Portion of pycnium, *e*, and very young aecium, *h*, and the mycelium between. Lower epidermis at *i*. Binucleate cells at the inner end of the pycnium, *a, b*, and in the mycelium, *c, d, f, g*. $\times 640$.

B.—(Agr.) Young aecium from 13-day fertile infection. Binucleate and trinucleate cells at *a, b, c, e*. Lower epidermis at *d*. $\times 640$.

C.—(Agr.) Ten-day fertile infection. Cells of lower half of aecium enlarging, *b, c*. Binucleate cell at *a*. $\times 730$.

D.—(Trit.) Fourteen-day infection. Paraphyses fixed a few minutes after mixing pycnosporos. Nuclei or nucleoluslike bodies at *a, b, c*. $\times 1,020$.

(pl. 13, C, *a, b, c*). In Plate 14, A, drawn from the same infection that contains the tissues shown in Plate 13, B and C, is represented the area between two aecia. The edge of one aecium is drawn at *a* and of the second at *e*. In between is to be found an abundance of diploid mycelium. There are mycelial cells with 2, 3, 4, and even 5 nuclei. (Pl. 14, A, *b, c, d, f, g, h*.) The word "diploid" has been retained here, but whether it is apposite under such circumstances might be questioned.

In Plate 13, D, is a small diagram of an aecium from an infection fixed three days after transferring pycniospores to it, and in Plate 14, B, the upper half of the same aecium is drawn enlarged. Under low power it can not be distinguished from the corresponding stage of development of the sterile aecium (cf. pl. 9, A, and pl. 13, D), but under high power the difference is marked (cf. pl. 9, B, and pl. 14, B). The sterile aecium is composed of gametophytic cells, while the fertile aecium is a mixture of gametophytic and sporophytic cells.

There are now more nuclei in the centrally located sporophytic cells. Earlier (pl. 13, B) 2 nuclei in a cell were common, 3 or 4 in a cell exceptional. There are still binucleate cells, but now 3 and 4 nuclei in a cell are common (pl. 14, B, *d, f, h*), and the cell at *b* contains 7. As before, there are binucleate cells in the marginal areas (*a, c, i, j*) and in the sterile tissue (*g*). This increase in the number of nuclei per cell would seem to be due to nuclear divisions. No indication of cell fusions has been noted.

There is here the beginning of another change. Near the line of demarcation between the sterile and fertile areas of the aecium (pl. 14, B, *e*) the intercellular spaces are becoming filled with a homogeneous substance, perhaps derived from the breakdown of the walls of the sterile tissue.

The multinucleate cells now elongate, pushing out into the sterile tissue as they grow. Plate 15, A, *f* and *g*, shows an early step in this process, while *b, c*, and *e* are somewhat more advanced. Marginal multinucleate cells, as at *a*, sometimes develop later than the central ones, while more remote cells, as at *i*, may take no share in this activity. In this marginal area the cytoplasm of the cells is thin, and many of the nuclei are indistinct.

Material fixed four days after mixing pycniospores of different infections on a leaf shows all stages in the aecia, from the multinucleate cells of Plate 15, A, to young spore chains. In Plate 15, B, is drawn a small group of multinucleate cells slightly older than those above. Here are elongated cells containing 8 or 10 nuclei, with the oldest (pl. 15, B, *c, f*) shaping toward cell division. Coming up between the larger cells are smaller ones with 4 or 5 nuclei (*b, e*), and still smaller ones with 2 (*a, d*).

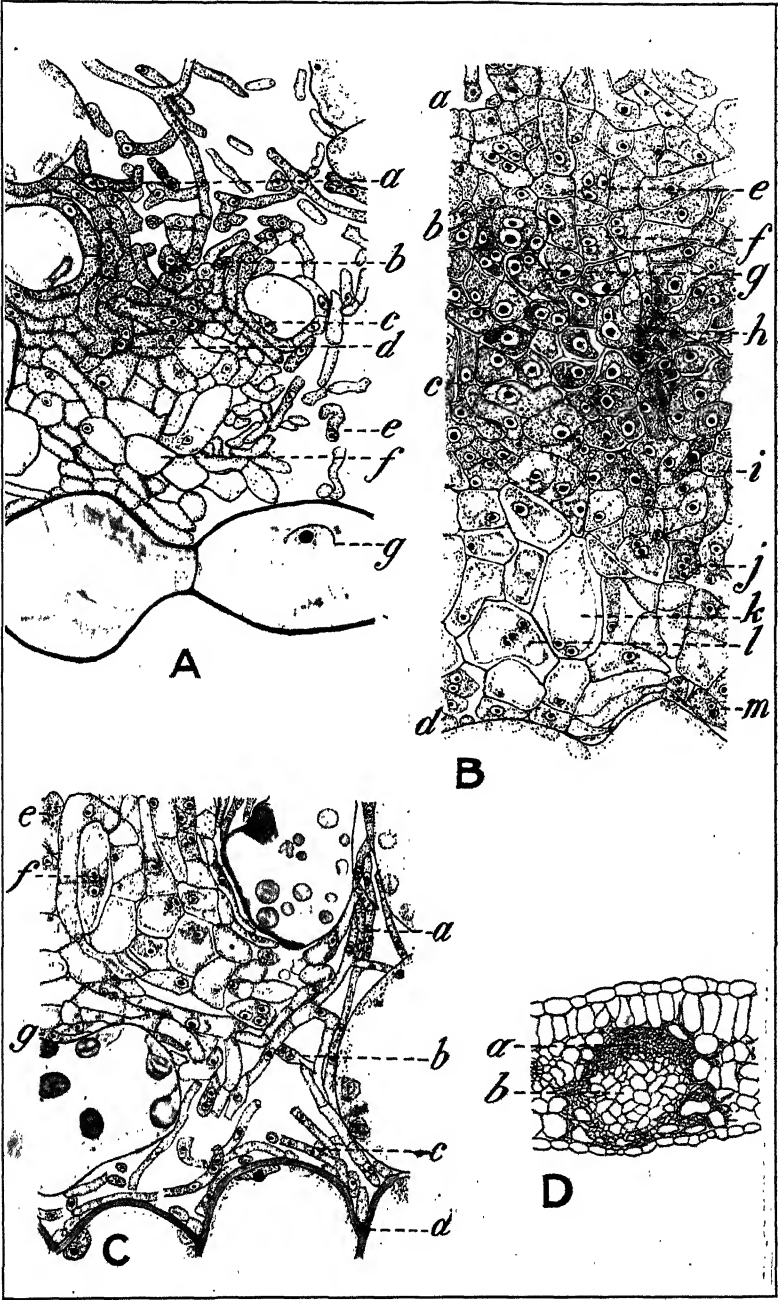
EXPLANATORY LEGEND FOR PLATE 13

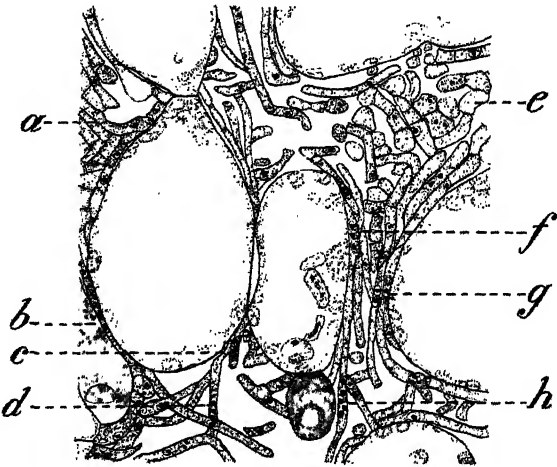
A.—(Agr.) Slightly later stage of development of fertile aecium from 10-day infection. Upper half, *a-d*, composed of small dense cells; lower half, *d-f*, of larger vacuolate cells. Sporophytic cells at *a, b, c, d, e*. $\times 730$.

B.—(Agr.) Narrow strip through older fertile aecium from 13-day infection. From *a* to *b* is the basal tissue; *b* to *c*, sporogenous area; *c* to *d*, sterile "space-making" area. Sporophytic cells at *c, f, g, h, i, j, k, l, m*. $\times 730$.

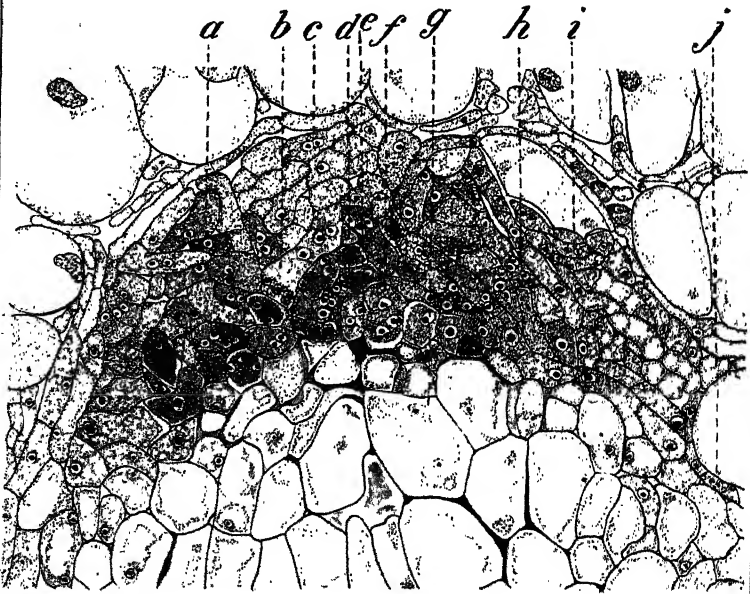
C.—(Agr.) Thirteen-day fertile infection. The lower corner of an aecium and adjoining mycelium showing presence of cells with 2, 3, or 4 nuclei at *a, b, c, e, f, g*. Lower epidermis at *d*. $\times 730$.

D.—(Agr.) Diagram of older fertile aecium, fixed 3 days after mixing the spores. Sporogenous tissue at *a* and large empty cells at *b*. $\times 115$.





A



B

Notwithstanding the fact that at this stage the tips of these multinucleate cells may contain 5 or 6 nuclei (pl. 15, B, c, f), the first cell division usually (not always) cuts off a binucleate cell. Very few terminal cells have been seen with more than 2 nuclei, and later cells are quite uniformly binucleate. In Plate 15, C, there is a young spore chain consisting of a basal cell, c, and two young binucleate spore mother cells, d, e. Soon after this the older spore mother cells divide, forming the spore and the intercalary cells. In Plate 15, C, b, the nuclei have divided, preparatory to cell division, and in Plate 15, D, the division into a large spore, d, and a small intercalary cell, c, is complete. The number of nuclei in the basal cell decreases as spore formation progresses, but occasionally, even after the young spore chains are well begun, there may still be 3 (pl. 15, C, a), 4 (pl. 15, C, c), or even 5 (pl. 15, D, a).

Plate 15, E, is a diagram of an aecium bearing young spores. The sporophytic growth, a, forms a hemispherical mass arching into the sterile area, b. In Plate 15, F, the spore chains and adjoining cells of the same aecium are drawn enlarged. The young spore chains here are well organized and the basal cells (pl. 15, F, b, e) are regularly binucleate. The inner end of the basal cell may be broad or narrow, squared or irregular. In other rusts, basal cells have been described as arising from the fusion of two cells, with the result that the cell presents a "2-legged" appearance. If such fusions occur in *Puccinia graminis* they are rare. The basal cell at Plate 15, F, e, might be so interpreted. Even in this case, however, the possibility is not excluded that it represents the branching of a basal cell rather than the union of two to form one. Of course fusions could occur undetected, since the two "legs" would not necessarily lie within the same section, but it is unlikely that if they occur regularly a few would not be in the plane of the section.

The breakdown of the sterile cells before the advance of the sporophytic tissue, the beginning of which was noted in Plate 14, B, e, and which is more evident in the later stage in Plate 15, A, d, is now pronounced. (Pl. 15, F, i.) A comparison of the three figures on this point suggests that it is not a mere matter of crushing the empty cells. Appearances suggest that the walls are being dissolved.

The spore chains now increase rapidly in both size and number and soon fill the whole interior of the aecium, taking the place of the sterile tissue of the younger aecium. On this account the sterile tissue has often been called "space-making tissue." The diameter of the aecium as a whole increases, as may be seen by comparing the diagrams in Plate 15, E, and Plate 16, A, which are drawn at the same magnification. The basal cells have elongated (pl. 16, A, a), and coming from each is a chain of 10 or 12 spores. The older spores are mature and lying loose within the cavity. The whole spore-bearing mass is enveloped in a peridial layer, b, derived on the sides from the peripheral spore chains and on the central arch from the terminal cells of the central chains. The expansion of the aecium has placed the leaf epidermis under strain, and the first break has occurred at c.

EXPLANATORY LEGEND FOR PLATE 14

A.—(Agr.) From a 13-day fertile infection, showing the edge of one aecium at a and of another at c, and the mycelium between. Cells with 2, 3, 4, or 5 nuclei at d, e, f, g, h. $\times 730$.

B.—(Agr.) Portion of Plate 13, D, enlarged. Cells with two or more nuclei at a, b, c, d, f, g, h, i, j. Beginning of breakdown of walls of space-making tissue at e. $\times 730$.

Plate 16, B, represents the basal cells and adjoining areas of Plate 16, A, enlarged. It is drawn to the same scale as Plate 15, A and F. Comparison shows a marked increase in the number of spore chains. In Plate 15, F, little cells from the base of the aecium (*a, d, f, g*) are elongating and forcing a narrow passage down between the established chains. That spore chains can arise from such beginnings is indicated by basal cells like those in Plate 16, B, *a, b*, and *c*, which take their origin from slightly deeper layers of the base of the aecium than the rest. The cells still deeper in the base of the aecium here are so far drained that the cytoplasm is vague and the nuclei ordinarily invisible. Only from younger stages can it be learned that many of these cells belong to the sporophyte generation.

The later history of the pycnium in infections bearing aeciospores differs markedly from that in sterile infections. When fertilization has taken place and aeciospores are developing, the production of pycniospores stops and the pycnial exudate dries. So certain is this that in working with living infections the drying of the epidermis above an infection can be counted on as proof that the diploid phase has begun and that aecia will open on the lower surface in a day or two.

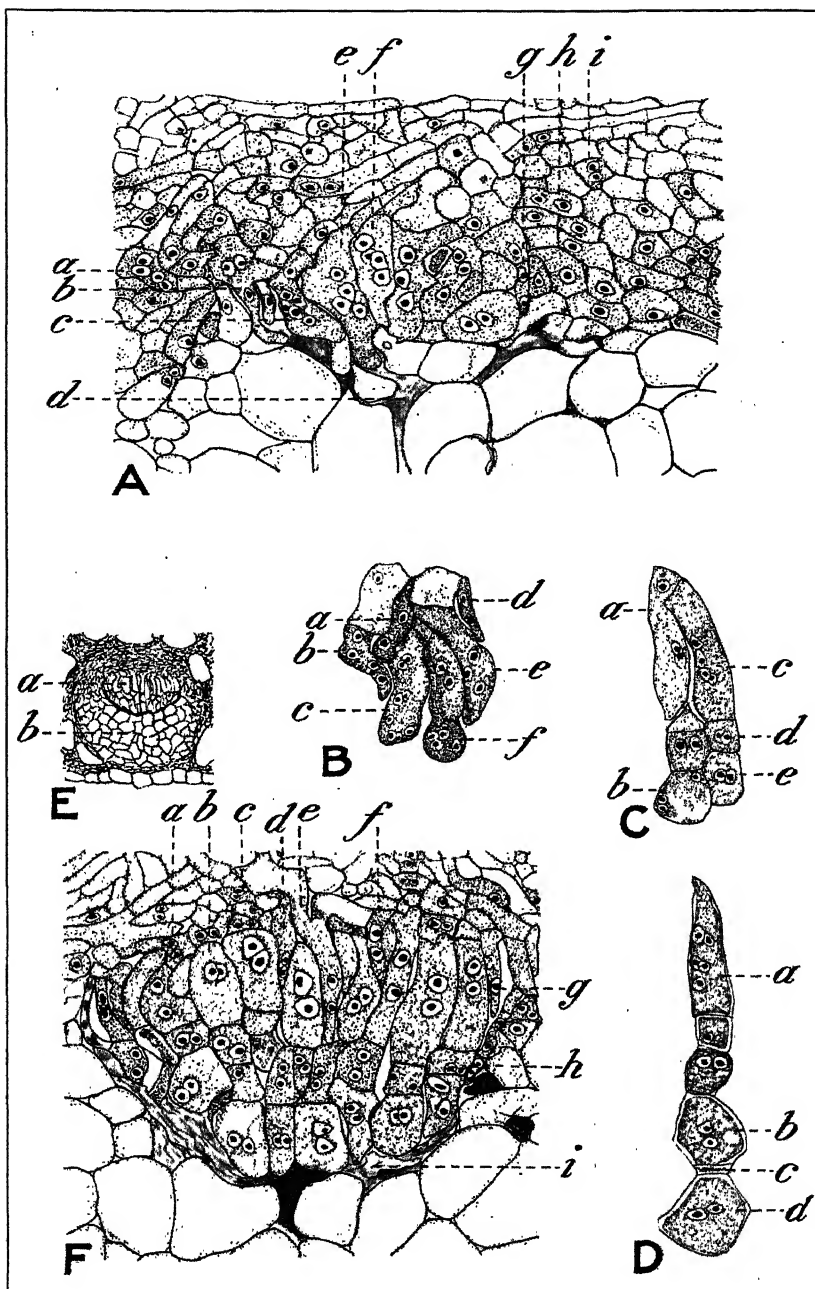
A cytological study of pycnia in infections that have been fertilized shows none of the secondary changes found in sterile infections. The primary paraphyses wither, but no secondary paraphyses form. The ostiole of the pycnium remains narrow and there is comparatively little thickening of the pycnial wall and none of the abnormal basal growth of the pycnium. There may still be old spores within the pycnial cavity, but a study of the tips of the pycniosporophores shows no evidence that new spores are forming. The pycniosporophores soon elongate, blocking the central cavity, and then die.

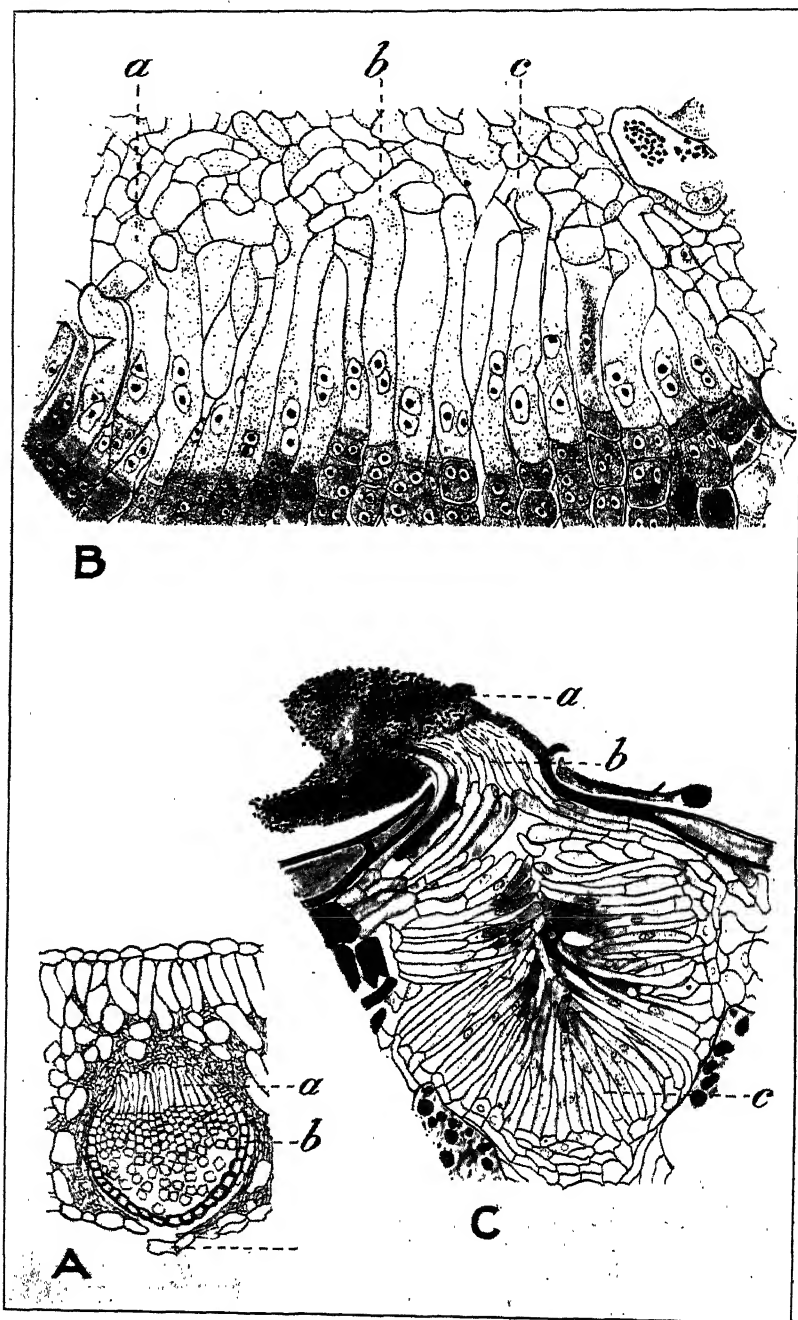
In Plate 17, A, is drawn a group of paraphyses from an infection several days after fertilization. In the 20-day infection from which this was drawn the aecia are soon to open and many of the pycnia have withered paraphyses. Here, however, the paraphyses are still living. The nuclear content is much the same as it was before fertilization. There are usually two larger, centrally located nuclei, *b, c*, and occasionally one (more rarely two) smaller bodies nearer the tip, *a*, which look like nuclei.

In Plate 16, C, is drawn a typical spent pycnium from an old (36 days) fertile infection. With its withered paraphyses, *b*, to which there still adheres a bit of dead, dry exudate, *a*, its narrow ostiole, and its pycniosporophores, *c*, elongated and dying, it forms a marked contrast to the much older but still living and active pycnium of the sterile infection. (Pl. 10, C.)

EXPLANATORY LEGEND FOR PLATE 15

- A.—(Trit.) Portion of aecium from 12-day fertile infection. Multinucleate cells, *c, e, f*, pushing out into sterile area. Breakdown of walls of sterile cells at *d*. Other sporophytic cells at *a, b, g, h, i*. $\times 730$.
 B.—(Agr.) Detail of sporogenous tissue from aecium 4 days after mixing pycniospores. Larger basal cells at *c* and *f*, smaller at *a, b, d, e*. $\times 730$.
 C.—(Agr.) Same material as B. Young spore chains showing multinucleate basal cells, *a, c*, and spore mother cells at *b, d, e*. $\times 730$.
 D.—(Agr.) Same material as B. Older spore chain, still with multinucleate basal cell, *a*. Spore mother cell at *b*, and spore, *d*, with intercalary cell, *c*. $\times 730$.
 E.—(Trit.) Diagram of fertile aecium of 12-day infection, showing young spore chains, *a*, and sterile tissue, *b*. $\times 115$.
 F.—(Trit.) Spore chains of E enlarged. Basal cells, *b*, and spore mother cells, binucleate. Younger basal cells at *d, f, g, h*. Possible fusion at *e*. Further breakdown of sterile tissue at *i*. $\times 730$.





IRREGULARITIES

The question arises whether *Puccinia graminis* ever reproduces homothallically. Haploid aecia of various ages have been canvassed for evidence. Occasionally an isolated infection bearing several haploid aecia will show a single fertile aecium. A study of the mycelium about its base usually shows the presence of one or more diploid cells, so it may be that here, too, the sporophyte generation arose before the aecium formed. The time, place, and method of its starting have not been traced. Without more information these sporadic fertile aecia in otherwise sterile infections can not be considered unquestionably homothallic.

One or two other irregularities have been found. In one case, two 36-day infections are in contact and their mycelia have interlaced, and both bear open aecia. Among the fertile aecia is one that is seemingly sterile. Under low power it looks like a typical old haploid aecium with large, nearly empty, dying cells. Under high power (pl. 17, B) there is found a central patch of three or four cells whose intervening walls are disappearing. Nuclear divisions have occurred, and the cytoplasm is far richer than in the surrounding cells. The preceding and following sections show only large dying cells like the ones surrounding the patch in the drawing. This seemingly haploid aecium, several weeks old and nearly dead, has suddenly formed by cell fusion the beginning of a sporophyte.

This occurred where two mycelia of opposite sex had interlaced. It is possible that this haploid aecium was composed of hyphae of both mycelia and that the cells which fused belonged to different sexes. Why, on such a basis, the fusion would be so long delayed is not clear; but, at any rate, this too can not be considered unquestionably homothallic.

Another irregularity is depicted in Plate 17, C. In this case two mycelia of the same sex overlapped and both remained sterile for several weeks. Then one of the 14 aecia formed an abnormal sporophytic growth. (Pl. 17, C.) It is too late to trace its origin, but there are several binucleate cells in the margin. (Pl. 17, C, a.) Very irregular multinucleate cells occur at the center (b, d), some of which are dying (e). There is an apparent attempt at spore formation, c being an abortive spore. It is doubtful whether normal spores could form. Both the fungus and its host are far spent.

DISCUSSION

The germination and entry of the sporidium of *Puccinia graminis* differ from the corresponding stages in the uredinal generations. The urediniospore forms a long germ tube which grows to a stoma and passes through into the substomatal air chamber. The mycelium is intercellular from the beginning. The sporidium pushes out a short beaklike protrusion which pierces the outer epidermal wall directly. Its first life in the host is intracellular.

EXPLANATORY LEGEND FOR PLATE 16

A.—(Trit.) Twelve-day fertile infection. Diagram of a nearly mature fertile aecium with elongated basal cells at a, peridium at b, and broken epidermis at c. $\times 115$.

B.—(Trit.) Portion of A. at a, enlarged, showing elongated binucleate basal cells, sometimes with irregular bases, a, b, c. $\times 730$.

C.—(Agr.) Old dead pycnium from a fertile 36-day infection. Withered primary paraphyses at b, with adherent dead exudate, a. Dying or dead elongated pycniosporophores at c. $\times 460$.

Perhaps correlated with this is the fact that in the efforts of the fungus to get through the very compact palisade layer of the leaf the intracellular growth there too is more or less mycelial in character. Even the haustoria formed later in mesophyll cells may be made up of more than one cell. It is probable, however, that all of these intracellular growths, whether haustorial in form or intermediate between that and ordinary hyphae, are haustorial in function and serve to extract food from the host. Perhaps here, too, as has been claimed for the uredinal generations of corn rust (47), the mycelium absorbs some food directly through hyphae, in addition to what it takes through specialized intracellular organs.

It was hoped at the outset of this work that traces of entry would persist and be detectable in older infections, so that if two or more entries occurred close together and the mycelia became intimately mixed it would still be possible to prove the double or multiple origin. Unfortunately, it is rare to find traces of primary hyphae after the fifth or sixth day. This adds an element of uncertainty in interpreting results. If an infection, supposedly single, and covered to exclude insects, develops fertile aecia, there is always the doubt whether it started as one or as two. Sometimes a careful cytological study resolves the doubt, but sometimes it can not be decided with certainty. In counting infections on the living host plant, a higher percentage develops open aecia than would be expected theoretically. The existence of doubles that look like singles, and trebles that look like doubles, is doubtless a factor in this discrepancy.

In *Puccinia graminis* it would appear that the sporophyte generation does not start in the aecium, nor in the mycelium (at least not commonly), but in the pycnium, and that pycniospores from another and different infection are necessary to its inception. The pycnium can no longer be considered functionless, for it has a vital function, in heterothallic rusts at least. Nor can it be considered a vestigial male organ, for, as Craigie (19) has pointed out, half of the pycnia are of one sex and half of the other, although they look alike.

In isolated unisexual infections of *Puccinia graminis* the pycnia continue to produce pycniospores as long as the fungus lives. When the primary paraphyses wither, a second and larger crop of paraphyses is produced. In infection in which fertilization has taken place, on the contrary, the pycnia stop forming spores, the pycniosporophores elongate and block the pycnial cavity, the primary paraphyses wither, no secondary paraphyses form, and the whole pycnium soon dies. The contrasting behavior in the two cases is striking. The pycnium has been assumed to be functionless, but this picture, taken as a whole, is not what would be expected of a functionless organ.

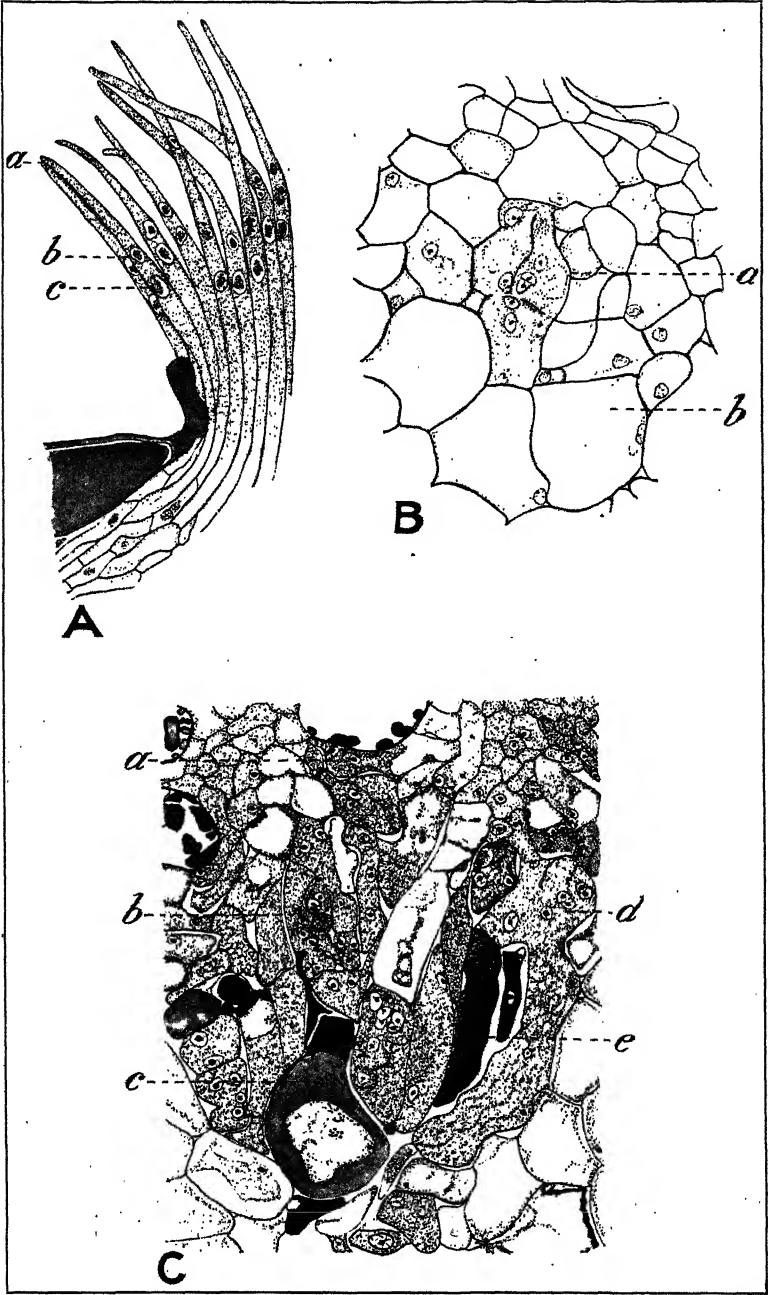
The gametophytic component of the aecium forms regularly, with or without the advent of the sporophyte generation. Evidently no stimulus connected with the presence of diploid cells is necessary for

EXPLANATORY LEGEND FOR PLATE 17

A.—(Agr.) Paraphyses of pycnium of 20-day infection, several days after fertilization. They contain usually two nuclei, b, c, with sometimes an additional smaller one, a. $\times 730$.

B.—(Agr.) Fusion of several cells at a in the center of an old haploid aecium of a 26-day fertile infection. Surrounding cells, b, nearly dead. $\times 1,020$.

C.—(Agr.) Abnormal young sporophytic growth in one of two old overlapping sterile infections. Binucleate cell at a. Multinucleate at b, d, e. Abortive spore at c. $\times 730$.



FOR EXPLANATORY LEGEND SEE PAGE 606

its formation. This has its parallel in other groups of fungi. Derx (21) found haploid sterile perithecia in monosporous cultures of *Penicillium*. Dodge (27) found "sterile bodies characteristic of haplont mycelia" in *Neurospora*. Mounce (39) describes the formation in a few cases of shallow pores on haploid mycelia of *Fomes pinicola*. And Hanna (32) found in *Coprinus lagopus* that haploid mycelia can produce imperfect fruit bodies. A few produce spores which are all of the same sex as the parent mycelium.

Just how widespread heterothallism is among rusts is at present a matter of conjecture only. Judging by the rapidly increasing list of heterothallic species in other groups of fungi, it will be found to be fairly common. It has long been noted that in both long-cycle and short-cycle rusts, pycnia appear regularly before the onset of the sporophyte generation. This fact now gains added significance. It may indicate that the pycnium of other rusts is functioning as it does in *Puccinia graminis*. Comparatively few rusts lack the pycnium entirely.

In the long list of rusts that have been studied cytologically, the majority have been described and figured as showing the origin of the sporophyte in the young growing aecium. There the haploid cells of the sporogenous area either fuse two by two (14) or the nucleus of one of a pair of cells migrates into the other (3). The binucleate cells so formed give rise to the spore chains. In some of the short-cycle rusts the sporophyte originates in a similar fashion in the primary uredinal sorus or in the telial sorus. The following list has been compiled:

- Blackman, 1904 (3).—*Phragmidium violaceum* Wint.
 Christman, 1905 (14).—*Phragmidium speciosum* Fr., *Caeoma nitens* S., *Uromyces caladii* Schw.
 Blackman and Fraser, 1906 (4).—*Uromyces poae* Raben., *Melampsora rostrupi* Wagn., *Puccinia poarum* Niels.
 Christman, 1907 (15).—*Phragmidium potentillae canadensis* Diet.
 Olive, 1908 (43).—*Triphragmidium ulmariae* (Schum.) Link, *Gymnoconia interstitialis* (Schlecht.) Lagerh.; *Phragmidium potentillae canadensis* Diet., *Chrysomyxa abietes* (Wallr.) Ung., *Puccinia transformans* E. and E.
 Dittschlag, 1910 (23).—*Puccinia faleariae* Pers.
 Hoffmann, 1912 (35).—*Endophyllum sempervive* (Alb. and Schw.) De Bary.
 Fromme, 1912 (29).—*Melampsora lini* (Pers.) Desm.
 Fromme, 1914 (30).—*Puccinia claytoniata* Peck., *P. violae* (Schum.) DC., *P. angustata* Peck, *P. hydrostylis* (Link) Cke., *P. eatoniae* Arthur, *Uromyces caladii* Farlow.
 Colley, 1918 (16).—*Cronartium ribicola* F. de Waldh.
 Adams, 1920 (1).—*Peridermium comptoniae* Orton and Adams, *P. pyriforme* (Peck) Hedge. and Long, *P. acicolum* Underw. and Earle, *P. peckii* Thüm., *P. balsameum* Peck.
 Thurston, 1923 (57).—*Gymnosporangium bermudianum* Farlow.
 Lindfors, 1924 (38).—*Melampsora reticulata* Blytt., *Coleosporium euphrasiae* (Schum.) Wint., *Uromyces acelosae* Schrot., *Puccinia tragopogi* (Pers.) Cda., *Triphragmidium ulmariae* (Schum.) Link, *Trachyspora alchemilliae* (Pers.) Fuck., *Puccinia northieri* Koern., *Chrysomyxa abietes* (Wallr.) Ung.
 Petry and Hutton, 1924 (46).—*Dicaeoma distichlidis* (Ellis and Ev.) Kuntze.
 Walker, 1927 (60).—*Puccinia cryptotaeniae* Peck, *P. xanthii* Schw., *P. fusca* (Pers.) Relb.

The above list is not complete but suffices to show the range. These rusts would appear at first sight to be homothallic. Even here, however, the possibility is not excluded that in some cases the haploid tissue in the young sorus includes (+) and (−) hyphae of separate origin and that a sorus without both elements would remain sterile.

Dodge (24, 25) found a situation in the short-cycle orange rust of *Rubus* (*Caeoma nitens* Burrill) that is suggestive. Some strains of the rust produce no pycnia, there are no cell fusions in the base of the aecium, the aeciospores formed are for the most part uninucleate, and the aeciospores germinate by forming 2-cell promycelia with two uninucleate sporidia. Other strains of the short-cycle rust produce pycnia, cell fusions occur in the base of the aecium, and binucleate aeciospores are produced which on germination produce (probably without either nuclear fusion or reduction) 4-cell promycelia with four sporidia. Heterothallism and the agency of the pycnium in fertilization here are probable, but the details of the process must differ from those in *Puccinia graminis*, for fusions are delayed until the young aecium has formed.

Although the majority of rusts that have been studied cytologically show cell fusions in the aecium or in the corresponding sorus in short-cycle forms without aecia, instances are not lacking in the literature in which binucleate hyphae are present at an earlier stage. Blackman and Fraser (4, p. 40) saw paired nuclei in the aecium in *Puccinia poarum* Niels. "before the differentiation of the fertile layer, or in cells below that layer after its differentiation." Olive (45) saw mixed perennial gametophytic and sporophytic mycelia in several species and found that in *Puccinia podophylli* Schw. aecia may arise from preexisting binucleate hyphae. Lindfors (38, p. 16) studied the development of aecia of *Puccinia tragopogi* (Pers.) Cda. in very young leaves and found that—

Es konnte konstatiert werden, dass die Zellen in fertilen Teil in weitem Masse mehrkernig waren, und dass dieser Zustand schon in denjenigen Hyphen herrschte, die sich von aussen an die Äzidienanlage anlegten.

In short-cycle rusts the binucleate condition often arises before the formation of basal cells in the sorus and even in the mycelium outside. This has been reported in *Puccinia malvacearum* Mont. by Blackman and Fraser (4), and of the microforms *Uromyces scillarum* Wint. and *Puccinia adozae* DC. they say (4, p. 43): "The general vegetative mycelium showed conjugate nuclei." In *Uromyces ficariae* Lév. (4, p. 43), another microform, the general mycelium was uninucleate but "the mass of mycelial hyphae round the teleutospore sorus, as well as those directly connected with teleutospore formation, appear to have conjugate nuclei." Lindfors (38) found widespread binucleate mycelium in *Puccinia albulensis* Magn., *P. epilobii* DC., *P. gigantea* Karst., *P. holboellii* (Hornem.) Rostr., *P. sari-fragae* Schlecht., and *Uromyces solidaginis*. Walker (60) found a similar condition in *Puccinia asteris* Schw. and *P. cryptotaeniae* Peck.

The probability is that some of these short-cycle rusts will be proved to be heterothallic. This can not be taken for granted, however, for there is an alternative. Lindfors (38) finds that in *Puccinia arenariae* (Schum.) Wint., a lepto-form, the teliospore gives rise to a 2-cell promycelium with two binucleate sporidia. The mycelium is binucleate and produces teliospores directly without cell fusions. This is certainly a homothallic species. Further study of these short-cycle rusts is needed to show whether the binucleate mycelium arises in connection with the pycnium (heterothallism) or in the promycelium (homothallism).

The evidence at hand as to the mode of origin of the sporophyte generation in *Puccinia graminis* is suggestive but not conclusive.

Without further supporting evidence, neither the fusion of two pycniospores nor the fusion of a pycniospore with a paraphysis can be considered proved. Neither would be without parallel in other fungi, for in the basidiomycetes it is believed that binucleate mycelium can arise by the fusion of two oidia, by the fusion of an oidium with a hypha, or by the fusion of two hyphae.

The latter possibility should be considered. When (+) and (-) mycelia interlace, it may be that the sporophyte can arise directly by the fusion of (+) and (-) mycelial cells. A careful search has not disclosed this in *Puccinia graminis* but it may occur none the less. It would be easily overlooked. Other workers on rusts have assumed that a fusion of vegetative cells may take place, and its occurrence in rusts is made more probable by the fact that it is known to occur in some smuts (33) and would appear to be of general occurrence in basidiomycetes.

Still another possibility should be considered. When two or more mycelia interlace, it is at least theoretically possible that (+) and (-) hyphae should contribute to the formation of the same pycnium and that both elements should form spores within the same pycnial cavity. There is no way in which this can be either proved or disproved, as (+) and (-) elements look alike. In such a case fertilization might occur within the pycnium without the introduction of spores from without.

In the aecium of *Puccinia graminis* the sporogenous cells are regularly multinucleate, and this condition is brought about, so far as noted, by rapid nuclear divisions. Multinucleate cells in the fertile layer of the young aecium have been reported in other rusts, but they have usually been ascribed to multiple fusions. Several workers have recorded the existence of an occasional trinucleate basal cell which gives rise to a chain of trinucleate spores (3, 4, 43, 23, 35, 29, 30, 16, 38). Basal cells with more than three nuclei also occur (16, 60, 38, 57, 43, 44), but have usually been regarded as of exceptional or unusual occurrence. Colley (16, p. 652) noted, however, that in *Cronartium ribicola* basal cells with more than two nuclei often give rise to binucleate spores, and he states:

Emphasis should perhaps also be placed on the constant and normal occurrence of multinucleate cells at the base of the aecium, * * *, by suggesting that in deep-seated aecia of caulicolous Peridermia the aeciospore chains may be found to arise more often from these placentalike cells than from basal cells arising from the fusion of only two cells in the fertile layer.

Olive (44) states:

Further, a large proportion of the 50 species of aecidium cups under investigation have been found to show a multinucleated stage in their development.

The discovery by Craigie of heterothallism in *Puccinia graminis* suggests that two physiologic forms of the rust growing together on a barberry might hybridize there and that new forms of the rust might result. This would complicate the question of breeding for rust resistance and give added impetus to the work of barberry eradication. The hybridizing of physiologic forms of smuts under controlled conditions has already been achieved (36, 31), and interspecific hybrids have been grown between two smuts (22) and between two ascomycetes (28).

New forms of the rust also may be found to arise by mutation, for mutations have occurred in the cultures of many fungi (55, 58, 33, 41,

37, 40, 7, and perhaps 56). One mutation in rusts has already been discovered by Newton and Johnson (42) in *Puccinia graminis*. It is possible that the teratological growth of the pycnia found in a single lot of material of *P. graminis* (pl. 10, B) is due to a mutation. It would be impossible to prove it, however, for it was discovered under the microscope after the material had been fixed.

In several of the mutations cited above, the mutants showed altered sexual behavior. The possibility should be kept in mind that some of the unexplained cases in *Puccinia graminis* in which a single fertile aecium appeared in a unisexual infection may be due to a localized mutation in the mycelium followed by a fusion between the mutant and the parent mycelium.

There is no positive evidence at present of more than two sexual groups in *Puccinia graminis*, but in so large and varied a species, with its numerous physiologic forms, it would not be surprising if other sexual groups were discovered.

SUMMARY

The sporidium of *Puccinia graminis* on a barberry leaf germinates, pierces the outer wall, and enters the epidermal cell, forming there a primary hypha of four to six cells.

From each cell of this primary hypha a branch grows down to the subepidermal area and develops into haploid mycelium. Intercellular hyphae are vigorous and shapely. A few intracellular hyphae form, but they are stunted, misshapen, and short-lived.

Several entries may occur close together, and the mycelia may interlace. No unmistakable case of fusion of vegetative cells has been noted. Young interlaced mycelia remain haploid.

The primary hypha degenerates rapidly and disappears.

Pycnial development begins on the fourth day. Hyphae grow up between palisade cells and form a mat between the epidermal and palisade layers. Branches converge at a common point. Large central upright hyphae lift the epidermis and serve as buffers against its pressure. The pycnium soon becomes organized into an outer wall from which slender-tipped pycniosporophores grow into the central cavity. Each pycniosporophore gives rise to a succession of spores. From around the upper edge of the wall, paraphyses grow in, meet, and turn outward, piercing the epidermis and forming a brush of tapering, stiff-looking hyphae outside of the epidermis. Each paraphysis contains 1, 2, or 3 nuclei.

An isolated unisexual infection produces from 3 or 4 to 2 dozen or more haploid aecia. These attain considerable size, undergo the first differentiation into areas, then become impoverished and die.

Pycnia in sterile infections remain active as long as the host tissues can supply food. When the primary paraphyses wither, secondary paraphyses are formed. Spores continue to be formed in abundance, and a drop of pycnial exudate is maintained on the surface of the leaf.

After pycniospores of one sex have been transferred to an infection of opposite sex, small granules which may be nuclei of pycniospores are found in the paraphyses. Later, cells with two or three nuclei are found in the wall of the pycnium and in hyphae leading from it.

Between a pycnium and a young fertile aecium are many haploid and a few diploid hyphae. Between the successively formed aecia

of a fertile infection there are sporophytic cells with two to four nuclei. Beyond the youngest aecium in the marginal mycelium is an occasional sporophytic hypha.

The gametophytic component of the aecium forms as in the sterile infection, but scattered throughout it from its very inception are sporophytic cells. These cells may contain 2, 3, or 4 nuclei.

Three days after pycniospores were transferred to an infection aecia have attained considerable size and the sporogenous area is well defined. The number of nuclei in the sporophytic cells in this region increases, apparently by nuclear divisions. Cells with three to six nuclei are common.

On the fourth day after fertilization these multinucleate cells push down into the sterile "space-making" tissue of the aecium to produce spore chains. Just before the first division a multinucleate cell contains 8 or 10 nuclei. These extra nuclei are utilized in forming the binucleate spore-mother cells so that the number in the basal cell steadily decreases. In well-established spore chains basal cells are regularly binucleate.

Soon after the advent of the sporophytic generation in an infection the pycnia stop forming spores and the pycniosporophores elongate, blocking the pycnial cavity. The paraphyses wither and no secondary paraphyses form. Pycnia of fertile infections die while the rest of the infection is still living and active.

A few cases of irregular and often abortive development in old haploid aecia have been noted. Their origin and nature are not fully known but may be homothallic in character.

LITERATURE CITED

- (1) ADAMS, J. F.
1920. II. SEXUAL FUSIONS AND THE DEVELOPMENT OF THE SEXUAL ORGANS IN THE PERIDERMIS. Penn. Agr. Expt. Sta. Bul. 160: 31-77, illus.
- (2) BENSANDE, M.
1918. RECHERCHES SUR LE CYCLE ÉVOLUTIF ET LA SEXUALITÉ CHEZ LES BASIDIOMYCÈTES. 156 p., illus. Nemours.
- (3) BLACKMAN, V. H.
1904. ON THE FERTILIZATION, ALTERNATION OF GENERATIONS, AND GENERAL CYTOLOGY OF THE UREDINEAE. Ann. Bot. [London] 18: [323]-373, illus.
- (4) ——— and FRASER, H. C. I.
1906. FURTHER STUDIES ON THE SEXUALITY OF THE UREDINEAE. Ann. Bot. [London] 20: [35]-48, illus.
- (5) BLAKESLEE, A. F.
1904. SEXUAL REPRODUCTION IN THE MUCORINEAE. Amer. Acad. Arts and Sci. Proc. 40: [205]-319, illus.
- (6) ———
1915. SEXUAL REACTIONS BETWEEN HERMAPHRODITIC AND DICIOUS MUCORS. Biol. Bul. 29: 87-102, illus.
- (7) ———
1920. MUTATION IN MUCORS. Jour. Heredity 11: [278]-284, illus.
- (8) ———
1920. SEXUALITY IN MUCORS. Science (n. s.) 51: 375-382, 403-409.
- (9) ——— and CARTLEDGE, J. L.
1927. SEXUAL DIMORPHISM IN MUCORALES. II. INTERSPECIFIC REACTIONS. Bot. Gaz. 84: 51-57.
- (10) ———, CARTLEDGE, J. L., and WELCH, D. S.
1921. SEXUAL DIMORPHISM IN CUNNINGHAMELLA. Bot. Gaz. 72: [185]-219, illus.
- (11) ———, CARTLEDGE, J. L., WELCH, D. S., and BERGNER, A. D.
1927. SEXUAL DIMORPHISM IN MUCORALES. I. INTRASPECIFIC REACTIONS. Bot. Gaz. 84: 27-50.

- (12) BRUNSWIK, H.
1924. UNTERSUCHUNGEN ÜBER DIE GESCHLECHTS- UND KERNVERHÄLTNISSE BEI DER HYMENOMYZETENGATTUNG COPRINUS. 152 p. Jena. (Bot. Abhandl. Goebel, Heft 5.)
- (13) BURGEFF, H.
1924. UNTERSUCHUNGEN ÜBER SEXUALITÄT UND PARASITISMUS BEI MUCORINEEN. I. 135 p., illus. Jena. (Bot. Abhandl. Goebel, Heft 4.)
- (14) CHRISTMAN, A. H.
1905. SEXUAL REPRODUCTION IN THE RUSTS. Bot. Gaz. 39: [267]-275, illus.
- (15) ———
1907. THE NATURE AND DEVELOPMENT OF THE PRIMARY UREDOSPORE. Wis. Acad. Sci., Arts, and Letters, Trans. 15: [517]-526, illus.
- (16) COLLEY, R. H.
1918. PARASITISM, MORPHOLOGY, AND CYTOLOGY OF CRONARTIUM RIBICOLA. Jour. Agr. Research 15: 619-660, illus.
- (17) COUCH, J. N.
1926. HETEROTHALLISM IN DICTYUCHUS, A GENUS OF THE WATER MOULDS. Ann. Bot. [London] 40: [849]-881, illus.
- (18) CRAIGIE, J. H.
1927. EXPERIMENTS ON SEX IN RUST FUNGI. Nature 120: 116-117, illus.
- (19) ———
1927. DISCOVERY OF THE FUNCTION OF THE PYCNIA OF THE RUST FUNGI. Nature 120: 765-767.
- (20) ———
1928. ON THE OCCURENCE OF PYCNIA AND AECIA IN CERTAIN RUST FUNGI. Phytopathology 18: 1005-1015, illus.
- (21) DERX, H. G.
1925. L'HÉTÉROTHALLIE DANS LE GENRE PENICILLIUM (NOTE PRÉLIMINAIRE). Bul. Soc. Mycol. France 41: [375]-381.
- (22) DICKINSON, S.
1927. EXPERIMENTS ON THE PHYSIOLOGY AND GENETICS OF THE SMUT FUNGI.—HYPHAL-FUSION. Roy. Soc. [London] Proc., Ser. B 101: 126-136; 102: 174-176; 103: 547-555, illus.
- (23) DITTSCHLAG, E.
1910. ZUR KENNTNISS DER KERNVERHÄLTNISSE VON PUCCINIA FALCARIAE. Centbl. Bakt. [etc.] (II)-28: 473-492, illus.
- (24) DODGE, B. O.
1924. UNINUCLEATED AECIDIOSPORES IN CAEOMA NITENS, AND ASSOCIATED PHENOMENA. Jour. Agr. Research 28: 1045-1058, illus.
- (25) ———
1926. THE QUESTION OF NUCLEAR FUSIONS IN THE BLACKBERRY RUST, CAEOMA NITENS. Jour Agr. Research 32: 1003-1024, illus.
- (26) ———
1927. NUCLEAR PHENOMENA ASSOCIATED WITH HETEROTHALLISM AND HOMOTHALLISM IN THE ASCOMYCETE NEUROSPORA. Jour. Agr. Research 35: 289-305, illus.
- (27) ———
1928. UNISEXUAL CONIDIA FROM BISEXUAL MYCELIA. Mycologia 20: 226-234, illus.
- (28) ———
1928. PRODUCTION OF FERTILE HYBRIDS IN THE ASCOMYCETE NEUROSPORA. Jour. Agr. Research 36: 1-14, illus.
- (29) FROMME, F. D.
1912. SEXUAL FUSIONS AND SPORE DEVELOPMENT OF THE FLAX RUST. Bul. Torrey Bot. Club 39: 113-131, illus.
- (30) ———
1914. THE MORPHOLOGY AND CYTOLOGY OF THE AECIDIUM CUP. Bot. Gaz. 58: 1-35, illus.
- (31) GOLDSCHMIDT, V.
1928. VERERBUNGSVERSUCHE MIT DEN BIOLOGISCHEN ARTEN DES ANTHERENBRANDES (USTILAGO VIOLACEA PERS.). EIN BEITRAG ZUR FRAGE DER PARASITÄREN SPECIALISIERUNG. Ztschr. Bot. 21: 1-90, illus.

- (32) HANNA, W. F.
1925. THE PROBLEM OF SEX IN COPRINUS LAGOPUS. Ann. Bot. [London] 39: [431]-457.
- (33) ———
1929. STUDIES IN THE PHYSIOLOGY AND CYTOLOGY OF USTILAGO ZEAЕ AND SOROSPORIUM REILIANUM. Phytopathology 19: 415-441, illus.
- (34) ———
1929. NUCLEAR ASSOCIATION IN THE AECIUM OF PUCCINIA GRAMINIS. Nature 124 (3120): 267.
- (35) HOFFMANN, A. W. H.
1912. ZUR ENTWICKLUNGSGESCHICHTE VON ENDOPHYLLUM SEMPERVIVE. Centbl. Bakt. [etc.] (II) 32: 137-158, illus.
- (36) KNIEP, H.
1919. UNTERSUCHUNGEN ÜBER DEN ANTHERENBRAND (USTILAGO VIOLACEA PERS.). EIN BEITRAG ZUM SEXUALITÄTSPROBLEM. Ztschr. Bot. 11: [257]-284.
- (37) ———
1923. ÜBER ERBLICHE ÄNDERUNGEN VON GESCHLECHTSFAKTOREN BEI PILZEN. Ztschr. Induktive Abstamm. u. Vererbungslehre 31: 170-183.
- (38) LINDFORS, T.
1924. STUDIEN ÜBER DEN ENTWICKLUNGSVERLAUF BEI EINIGEN ROSTPILZEN AUS ZYTOLOGISCHEN UND ANATOMISCHEN GESICHTSPUNKTEN. Svensk. Bot. Tidskr. 18: 1-84, illus.
- (39) MOUNCE, I.
1929. STUDIES IN FOREST PATHOLOGY. II. THE BIOLOGY OF FOMES PINICOLA (SW.) COOKE. Canada Dept. Agr. Bul. 111, 74 p., illus.
- (40) NADSON, G., and PHILIPPOV, G.
1928. DE LA FORMATION DE NOUVELLES RACES STABLE CHEZ LES CHAMPIGNONS INFÉRIEURS SOUS L'INFLUENCE DES RAYONS X. Compt. Rend. Acad. Sci. [Paris] 186: 1566-1568.
- (41) NEWTON, D. E.
1926. THE BISEXUALITY OF INDIVIDUAL STRAINS OF COPRINUS ROSTRUPIANUS. Ann. Bot. [London] 40: [105]-128, illus.
- (42) NEWTON, M., and JOHNSON, T.
1927. COLOR MUTATIONS IN PUCCINIA GRAMINIS TRITICI (PERS.) ERIKSS. AND HENN. Phytopathology 17: 711-726, illus.
- (43) OLIVE, E. W.
1908. SEXUAL CELL FUSIONS AND VEGETATIVE NUCLEAR DIVISIONS IN THE RUSTS. Ann. Bot. [London] 22: [331]-360, illus.
- (44) ———
1910. THE PRESENT STATUS OF THE CYTOLOGY OF THE RUSTS. Science (n. s.) 31: 437-438.
- (45) ———
1913. INTERMINGLING OF PERENNIAL SPOROPHYTIC AND GAMETOPHYTIC GENERATIONS IN PUCCINIA PODOPHYLLI, P. OBTEGENS, AND UROMYCES GLYCRRHIZAE. Ann. Mycol. 11: [297]-311, illus.
- (46) PETRY, E. J., and HUTTON, L. D.
1924. CONJUGATION IN THE AECIUM OF DICAEOMA DISTICHLIDIS. (Abstract) Phytopathology 14: 33-34.
- (47) RICE, M. A.
1927. THE HAUSTORIA OF CERTAIN RUSTS AND THE RELATION BETWEEN HOST AND PATHOGENE. Bul. Torrey Bot. Club 54: 63-153, illus.
- (48) SATINA, S., and BLAKESLEE, A. F.
1925. STUDIES ON BIOCHEMICAL DIFFERENCES BETWEEN (+) AND (-) SEXES IN MUCORS. I. TELLURIUM SALTS AS INDICATORS OF THE REDUCTION REACTION. Natl. Acad. Sci. Proc. 11: 528-534.
- (49) ——— and BLAKESLEE, A. F.
1926. THE MUCOR PARASITE PARASITELLA IN RELATION TO SEX. Natl. Acad. Sci. Proc. 12: 202-207.
- (50) ——— and BLAKESLEE, A. F.
1926. STUDIES ON BIOCHEMICAL DIFFERENCES BETWEEN (+) AND (-) SEXES IN MUCORS. II. A PRELIMINARY REPORT ON THE MANOILOV REACTION AND OTHER TESTS. Natl. Acad. Sci. Proc. 12: 191-196.
- (51) ——— and BLAKESLEE, A. F.
1927. FURTHER STUDIES ON BIOCHEMICAL DIFFERENCES BETWEEN SEXES IN PLANTS. Natl. Acad. Sci. Proc. 13: 115-122.

- (52) SATINA, S., and BLAKESLEE, A. F.
1928. STUDIES ON BIOCHEMICAL DIFFERENCES BETWEEN (+) AND (-) SEXES IN MUCORS. IV. ENZYMES WHICH ACT UPON CARBOHYDRATES AND THEIR DERIVATIVES. *Natl. Acad. Sci. Proc.* 14: 229-235.
- (53) ——— and BLAKESLEE, A. F.
1928. STUDIES ON BIOCHEMICAL DIFFERENCES BETWEEN (+) and (-) SEXES IN MUCORS. V. QUANTITATIVE DETERMINATIONS OF SUGARS IN (+) AND (-) RACES. *Natl. Acad. Sci. Proc.* 14: 308-316.
- (54) STAKMAN, E. C., and CHRISTENSEN, J. J.
1927. HETEROTHALLISM IN USTILAGO ZEAЕ. *Phytopathology* 17: 827-834.
- (55) ———, CHRISTENSEN, J. J., and HANNA, W. F.
1929. MUTATION IN USTILAGO ZEAЕ. (Abstract) *Phytopathology* 19: 106.
- (56) STEVENS, F. L.
1928. THE SEXUAL STAGE OF FUNGI INDUCED BY ULTRA-VIOLET RAYS. *Science (n. s.)* 67: 514-515, illus.
- (57) THURSTON, H. W., JR.
1923. INTERMINGLING GAMETOPHYTIC AND SPOROPHYTIC MYCELIUM IN GYMNOSPORANGIUM BERMUDIANUM. *Bot. Gaz.* 75: 225-248, illus.
- (58) VANDENDRIES, R.
1924. NOUVELLES RECHERCHES SUR LA SEXUALITÉ DES BASIDIOMYCÈTES. *Bul. Soc. Roy. Bot. Belg.* 56: [73]-97, illus.
- (59) ———
1924. CONTRIBUTION NOUVELLE À L'ÉTUDE DE LA SEXUALITÉ DES BASIDIOMYCÈTES. *Cellule* 35: [129]-155, illus.
- (60) WALKER, R. I.
1927. CYTOLOGICAL STUDIES OF SOME OF THE SHORT-CYCLED RUSTS. *Wis. Acad. Sci., Arts, and Letters, Trans.* 23: [567]-582, illus.
- (61) WILCOX, M. S.
1928. THE SEXUALITY AND ARRANGEMENT OF THE SPORES IN THE ASCUS OF NEUROSPORA SITOPHILA. *Mycologia* 20: 3-17, illus.

THE ANATOMY OF EUPHORBIA INTISY¹

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INTRODUCTION

Among the many peculiar plants that characterize the arid region of southern Madagascar, one of the most interesting is *Euphorbia intisy*.² Not only does this plant present several characteristics that make it worthy of considerable study by the theoretical botanist, but its past performance as a rubber-producing plant makes it seem possible that it may prove adaptable to American conditions.

Euphorbia intisy was discovered June 7, 1891, at Tsivory, Madagascar (8, p. 107-109).³ Commercial exploitation followed at once, so that the annual rubber exportation of Madagascar rose from less than 50,000 pounds to almost a million pounds. The ruthless exploitation, however, soon showed its effect, for within a few years intisy had become so rare that Prudhomme (10) in 1899, even before the plant had been named botanically (4), reported that where intisy had formerly been very common it was already difficult to find specimens.

The manner of obtaining intisy rubber is very simple and is one of the factors that have led to the commercial disappearance of this plant. The latex, which exudes from any cut, coagulates in the air without further treatment. Hence all that the native has to do is to make cuts in the stem, return after a few hours or a few days and pull off bands of rubber of very high quality, which he wraps into balls.

The yield of rubber from stems less than 3 cm. in diameter is so slight that it does not pay to tap them. Consequently, at the present time, because of the disappearance of the large trees, one finds tapping scars only at the collar, the most favored tapping position. (Fig. 1.) It is reported, however (9), that during the time of its great exploitation spiral cuts were made all the way up the tree from the ground to branches as small as 2 or 3 cm. in diameter, 15 pounds or more of rubber being obtained at one tapping from a single tree. In many cases the first tapping was so severe that the tree died outright. Trees subjected to less severe tapping in some cases withstood several such onslaughts, but still the weakening—both structural and physiological—incident upon careless cutting, always brought about the death of the tree. From what is known about tapping other rubber plants and about the stem anatomy of this plant, intisy would almost surely be able to live for many years if careful tapping methods were employed.

That this valuable plant occurs throughout a large part of southern Madagascar, that it does not occur in pure stands, and that it requires

¹ Received for publication Oct. 10, 1929; issued April, 1930.

² This plant is variously known throughout the region where it grows as intisy, inty, herokazo, herobay, erobay, herotra, caoutchouc sans feuilles, caoutchouc du sud, caoutchouc Antandroy, kokomy, and pira Mahafaly. The rubber from it is referred to in commerce as kilwa, niggers, or intisy.

³ Reference is made by number (italic) to "Literature cited," p. 625.

a number of years before it reaches a size sufficient to tap for rubber, have combined to keep the species alive, even though as early as 1906



FIGURE 1.—General aspect of the root system of *Euphorbia intisy* showing the hydiorrhizas and the absorbing roots. Note also the tapping scar at the collar. $\times \frac{1}{4}$

it was reported that intisy rubber was becoming unobtainable on the market, in spite of its valuable qualities which made it greatly desired in the automobile industry (2, p. 231).

A small number of living specimens that reached France during the first five years of this century made possible a few anatomical descriptions, but intisy proved unadapted to life in French greenhouses. In 1928 it was believed by the authorities on Madagascar plants that *Euphorbia intisy* did not exist except in Madagascar; also, it was feared that the species had become extinct in Madagascar.⁴

One of the chief results of the Humbert-Swingle Madagascar plant-exploration expedition of 1928⁵ was the finding of *Euphorbia intisy* at several places in southern Madagascar and the bringing back to Tananarive, Marseille, Algiers, and Washington of living specimens of this plant. The description that follows is based upon material collected by the writer at Ranohasy, Ambondro, and Behara, Madagascar, in August and September, 1928, and upon material obtained in Washington from the living plants brought back from Behara. In the description the writer has also drawn upon the results of the French investigators who studied this plant.

CLASSIFICATION

Euphorbia intisy (*Euphorbia intisy* Drake), according to Denis (3), belongs to the subsection Tirucalli of the section Euphorbium. This subsection includes a number of tropical xerophytic trees and shrubs in which the process of photosynthesis is largely or even completely taken over by the stems, which in most cases are fleshy.

The following floral description is chiefly from Drake (4) and Fron (6):

Inflorescences are deep red, produced in summer (December to February), borne in short, fleshy terminal cymes with most of the flowers abortive. Flower-

⁴ A type distributed as *Euphorbia intisy* var. *mainly* Poisson had been found to be only a type of *E. laro* Drake.

⁵ Sponsored by the University of Algiers, the Arnold Arboretum of Harvard University, and the United States Department of Agriculture.

ing is rare and seems entirely confined to old plants. The sexes are separate, but it is still unknown (1930) whether *intisy* is monoecious or dioecious. Cyathia urceolate with five disciform glands; stamens in five groups of three or four each, arranged with articulate peduncle near the anther; ovary usually bilocular; styles bifid; capsule spherical, slightly flattened, 2 to 3 cm. in diameter; seeds hemispherical, smooth, brown, and ripening about three months after flowering and quickly losing their germinative power.

VEGETATIVE CHARACTERS

The writer has not seen either flowers or fruits of *Euphorbia intisy*, but in the field it is relatively easy to distinguish this plant from the other arborescent *Euphorbias* which make up the "intisy brush." Its chief characteristics are as follows: (1) Unlike any similar form,



FIGURE 2.—The largest known plant of *Euphorbia intisy* now alive. Photograph taken in the garden of the Chief Administrator, Ambondro, Madagascar, September 8, 1928

the branches are ligneous up to within a few millimeters of the growing point; (2) the youngest branches are of much smaller diameter than those of any similar *Euphorbia*; (3) the latex is not sticky, but when a bit of it is rubbed in the hand it soon coagulates and seems to contain nothing but pure rubber; and (4) the root system is unique. (See p. 620.)

Intisy is a shrub or small tree, reported to reach a height of 7 meters and a stem diameter of 25 cm. However, no trees of this size are known to exist to-day, the largest that the writer saw being approximately 4 meters high. (Fig. 2.) The stem is upright and frequently branched at the base, but it does not throw up suckers from below the surface of the ground even after the top has been entirely cut away—a point which has doubtless been of considerable importance in the economic disappearance of the plant. The general appearance

of the tree is usually unimposing and straggling. The young stems, however, are dichotomously branched and present a regular, graceful appearance, resembling mistletoe. The stem remains greenish and smooth for many years, the epidermis being covered only by a thick waxy covering. After many years a cork cambium is formed (in the hypoderm or deeper), after which the stem presents a scaly, brown appearance, due to the bark formed by this phellogen.

THE STEM

Intisy is virtually leafless, although in a rapidly growing state it has reduced sessile leaves, lanceolate or spatulate, narrowed at the

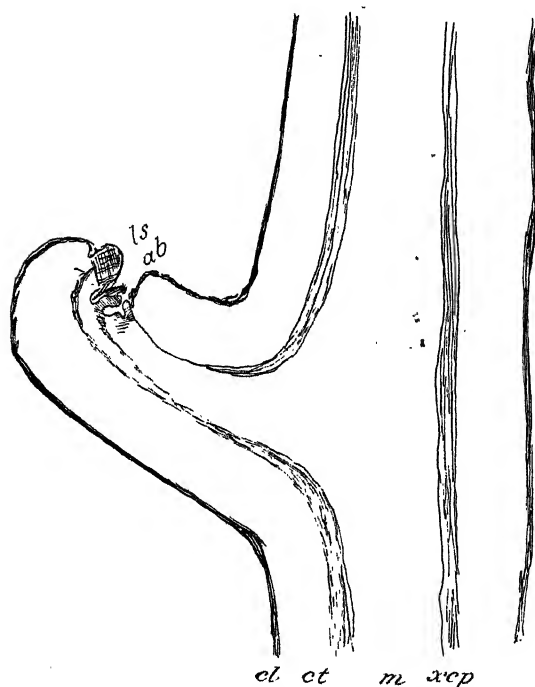


FIGURE 3.—Camera-lucida drawing, showing peculiar type of nodes displayed by certain plants of *Euphorbia intisy*: *p*, Phloem region; *ls*, leaf scar; *ab*, axillary bud; *cl*, cuticle; *ct*, cortex; *m*, pith; *x*, xylem; *c*, cambium. $\times 15$

base, 5 to 15 mm. in length and 1 to 2 mm. in width. Costantin and Gallaud (1) reported that the leaves were 30 by 2 mm., and Fron (6), 10 by 4 or 5 mm. Fron reported that the lower surface of the leaf was covered with multicellular, clavate hairs, but all leaves and growing points examined by the writer were found to be entirely glabrous. Stomata are sparse on both surfaces. A well-developed cuticle is present and the leaf has a glaucous appearance. The leaves and growing points are more or less red, the reddening being the more pronounced on shoots developed under cool conditions. Occupying the position of the stipules, which are wanting, a

gland lies on either side of the leaf at its insertion. In the greenhouse, even under very humid conditions, the leaves fall in a few days, so that a few centimeters behind the growing point no leaves are displayed.

The usual behavior is for a terminal bud to form after a few centimeters of growth. From this terminal, three or four branches (rarely one, two, or five) arise. Only occasionally do side buds grow out, most branching being of the dichotomous type described.

Some irregularity in phyllotaxy is displayed, but usually it is immediately obvious that the plant is alternate-leaved, having a $2/5$ arrangement. Each leaf receives from the stem a single bundle running the entire length of the leaf and giving off numerous lateral branches, which anastomose freely.

Some of the plants show a very peculiar type of emergence in connection with most of the nodes. As shown in Figure 3, the bud with its subtending leaf is borne on a projecting shoulder, which, while superficially resembling a short lateral branch, is not derived from the lateral bud, but is entirely the product of the stem. This unusual structure is found on approximately 30 per cent of the growing plants and on some of the preserved material obtained at Behara. It was not noted in the field and apparently was never observed by any of the early investigators who studied *intisy*, evidently not being present on the few plants previously available for study. Possibly this feature is of specific rank, though in other vegetative characters the plants seem to be like the ones that do not show this peculiar structure. Also, it is possible that the plants showing this structure are of the opposite sex from the others. At present, however, in the absence of flowers, such suggestions are little more than speculations.

Figure 4 shows in both longitudinal and transverse section the aspect of the young stem about 2 cm. behind the tip of a dormant branch. The outside is covered by a thick cuticle, and a hypoderm lies just beneath the epidermis. There is no evidence

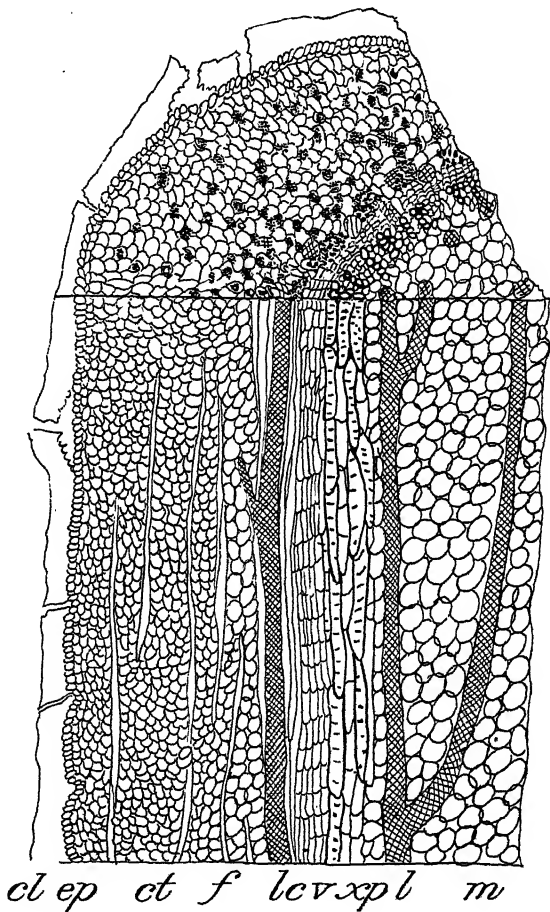


FIGURE 4.—Longitudinal and transverse section through young stem of *Euphorbia intisy*, just behind growing point, taken when dormant: v, Vessels; xp, xylem parenchyma; m, pith; cl, cuticle; c, cambium; ep, epidermis; cl, cortex; f, fibers; l, latex cells. $\times 80$

of a cork cambium, this being formed only after many years. Inside the hypoderm lies the broad cortical parenchyma, made up of palisade cells containing much chlorophyll and frequent starch grains. Toward the exterior the cells fit together tightly, so that intercellular spaces occur only among the innermost layers of the cortex. Scattered throughout this entire region lie numerous longitudinal fibers, isolated or in small groups.

A few latex cells are scattered through the cortex. However, most of the latex cells lie just within this region, and the fibers likewise are found most abundantly here in the phloem, occurring in groups of a dozen or more and almost completely setting off the cortical parenchyma from the phloem.

This arrangement of the fibers, radiating from groups (probably of pericyclic origin) lying next to the phloem, is very striking; according to Costantin and Gallaud (1), it is shown by no *Euphorbias* except the Madagascar representatives of the subsection *Tirucalli*. Moreover, the long fibers, which are either straight or branched, are characterized by not being compressed as are the cortical fibers of most *Euphorbias*. These fibers are made up of several layers of cellulose, the lumina becoming completely filled at a relatively young stage. Even in their later stages of growth, they remain almost entirely of cellulose. Treatment with hydrochloric acid and phloroglucin shows that the fibers contain but very little lignin, for they color but a faint pink.

The phloem is only slightly developed and appears almost identical with the cambium.

Within the cambium lies the well-developed xylem ring that characterizes the stem of *intisy*. This is made up of tracheids and vessels in the process of differentiation, interrupted tangentially by the vascular rays and radially by the xylem parenchyma. A large pith, made up of loosely fitting chlorophyllaceous cells larger than those of the cortex, occupies the center of the stem. There are no medullary fibers, but latex cells similar to those found outside the phloem run through the pith.

The latex cells have not been thoroughly investigated, but in all respects (except in the high rubber content of the latex) they seem to be similar to the latex cells of other members of the genus *Euphorbia* described by Gaucher (7). These latex cells consist of very long tubes without cross walls, branched but without anastomoses. The tubes have cellulose walls, evenly thickened throughout. Presumably in *intisy* also the tubes arise as individual cells in the embryo and continue to grow through the surrounding tissues, generally perpendicularly, throughout the life of the plant. They do not form lysigenous or by the linking up of individual cells, but only in the manner described, like the growth of fungus hyphae. Apparently latex cells never form anew either in wound tissue or from the cambium, though this point does not seem to have been thoroughly investigated.

In cross sections of unstained tissues the latex cells are sometimes difficult to distinguish from the fibers, but because of the fatty nature of the rubber which they contain, these cells stain red with Sudan III.

THE ROOT

The root system of *Euphorbia intisy* is apparently unlike that of any other plant. Figure 1 illustrates the general appearance of a part of the root system, showing the absorbing roots and the swollen, water-storage hydriarhizas;⁶ these, alternating with the short, constricted segments, give the entire root somewhat the appearance of a string of short, thick, link sausages. Although water-storage roots

⁶ "Hydriarhiza" is used here for the first time. From *hydria*, a water jug, + *rhiza*, a root, emphasizing the localized nature of the water-storage tissues.

are well known—e. g., *E. tuberosa* L.—the writer knows of none characterized by localized swellings such as these hydriarhizas of *E. intisy*.

Dubard and Viguier (5) have described the primary structure of the root, showing that the young tips of the hydriarhizas (erroneously called tubercules) and those of the absorbing roots are essentially identical and that no unusual anatomical characters are displayed in these portions.

Figure 5 shows the appearance of a portion of the root lying between two hydriarhizas. This root had undergone considerable secondary thickening, but of the usual sort and not of the anomalous type that characterizes the hydriarhizas themselves. Except for this radial thickening, the root structure at this point presents an aspect but slightly different from that of the stem shown in Figure 4. In the root, however, the cork cambium, which arises in the pericycle, has cut off considerable phelloderm. Owing to the expansion and occasional appearance of cross walls in the radial plane in this tissue, the serial arrangement of the phelloderm is soon lost, so that what is actually secondary tissue, derived from the cork cambium, appears almost identical with the primary cortex of the stem. It is not possible at this stage to locate the boundary between this phelloderm and the phloem or to tell which of the cells were part of the original pericycle.

The latex cells are slightly more numerous in the root than in the youngest stems, but in the root they all lie outside the cambium, none being present in the pithlike axial tissue.

Figure 6, which is drawn on the same scale as Figure 5, shows a section through one of the hydriarhizas on the same root, 2 cm. from the place where the transverse section shown in Figure 5 was made. The most striking point brought out here is the enormous enlargement that the cells of the axial tissue and the xylem parenchyma have undergone. In many cases these tissues have increased in volume a thousand times, yet apparently there has been no division of any of the cells lying inside the cambium. The walls of these aquiferous cells, which are stretched very thin, do not lignify.

Scattered through this region occur islands of xylem which have been forcibly pulled apart by the swelling of the intervening parenchyma cells. These constitute irregularly oriented strands, which, however, run in a general vertical direction.

Outside the cambium there has been little alteration in cell size, the cells having responded to the centrifugally exerted pressure by forming radial cross walls in the phelloderm and phellogen. The cells of the cork itself, being inelastic, are ruptured by this increase in girth, and characteristic cracks appear. (Fig. 1.) Apparently there is no increase in the number of fibers, for a given area in Figure 6 shows fewer fibers than a corresponding one in Figure 5. Also, as indicated in Figure 6, the fibers have been more or less displaced by the pressure exerted from within the cambium.

Gaucher (7) emphasized the fact that one of the characteristics of the Euphorbias is that the latex cells do not grow into the phloem ("liber"), a point which seems to hold with *intisy* for the stem. Undoubtedly, however, as Figures 5 and 6 show, latex cells do grow into the older phloem of the root, though not into the younger.

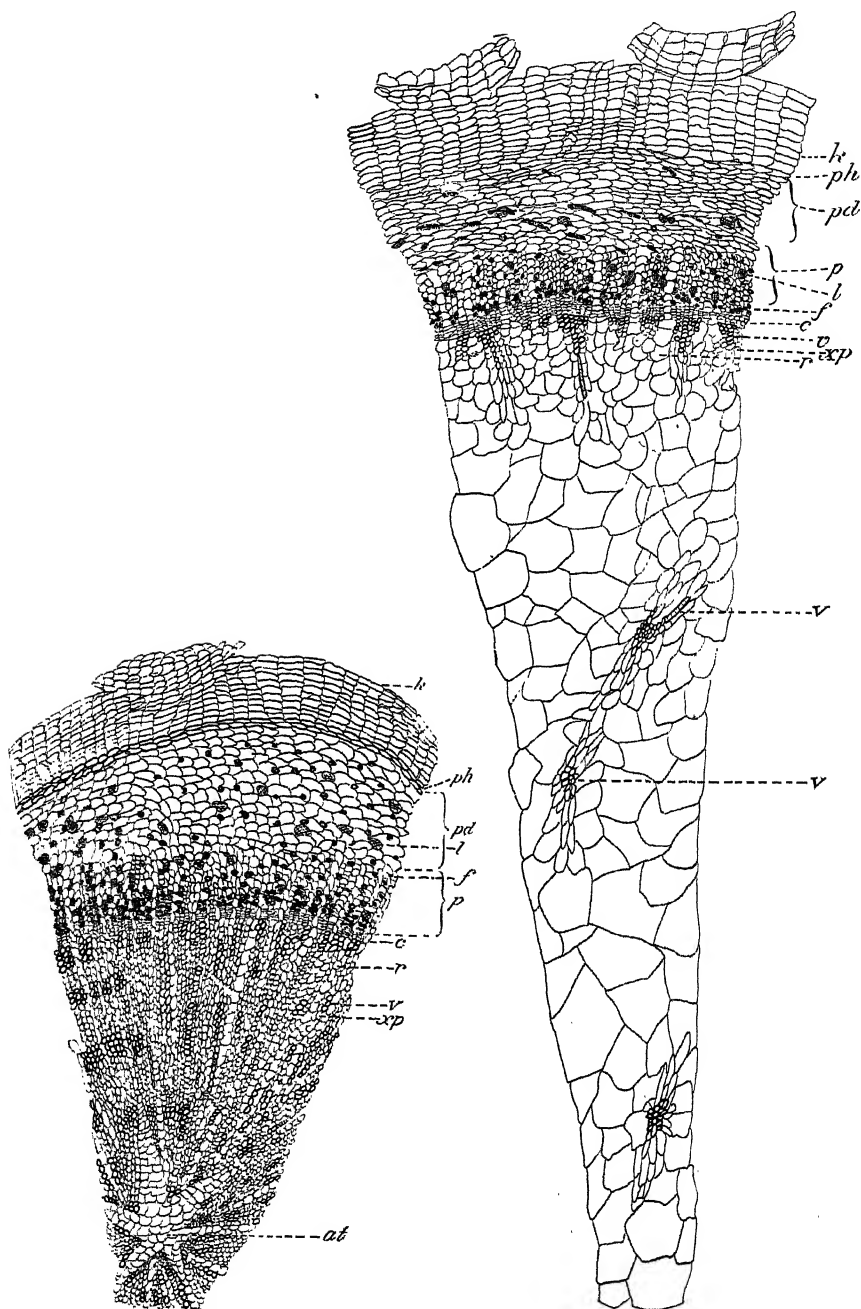


FIGURE 5.—Transverse section through unswollen portion of root of *Euphorbia intisy*: *c*, Cambium; *k*, cork; *ph*, phellogen; *pd*, phelloderm region; *l*, latex cells; *f*, fibers; *p*, phloem region; *r*, xylem rays; *r*, vessels; *xp*, xylem parenchyma; *at*, axial tissue. $\times 25$

FIGURE 6.—Transverse section through hydriarhiza of *Euphorbia intisy*. For explanation of letters see Figure 5. $\times 25$

Figure 7 shows the appearance of a dried root. The transverse plates so strongly indicated are the collapsed aquiferous tissue.

As Dubard and Viguier (5) pointed out, the anomalous swelling that leads to the production of these characteristic hydriarhizas commences early, usually with the appearance of secondary growth, the axial tissue undergoing considerable distension. This enlargement soon reaches into the ring of secondary xylem, the primary rays swelling, chiefly in the tangential plane. Frequently one of the six primary bundles fails to lignify, the cells of the bundle undergoing the same fate as those of the primary rays.

Next, all the secondary xylem rays enlarge, displacing the rows of vessels, and finally almost complete disruption is brought about when the xylem parenchyma cells, lying between the individual vessels, likewise enlarge. Except for the widely separated strands of vascular bundles that in cross section appear as scattered islands, the tissue appears almost homogeneous, and at this stage it is impossible to tell whether a given cell has been derived from an aborted primary vascular bundle, from axial tissue, from a primary or secondary ray, or from xylem parenchyma.

The aquiferous tissue is almost entirely water. The hydriarhizas are frequently consumed by the natives of Madagascar as a source of water, and the writer found that the slight taste of the watery pulp detracts but little from the value of these hydriarhizas to the thirsty traveler.

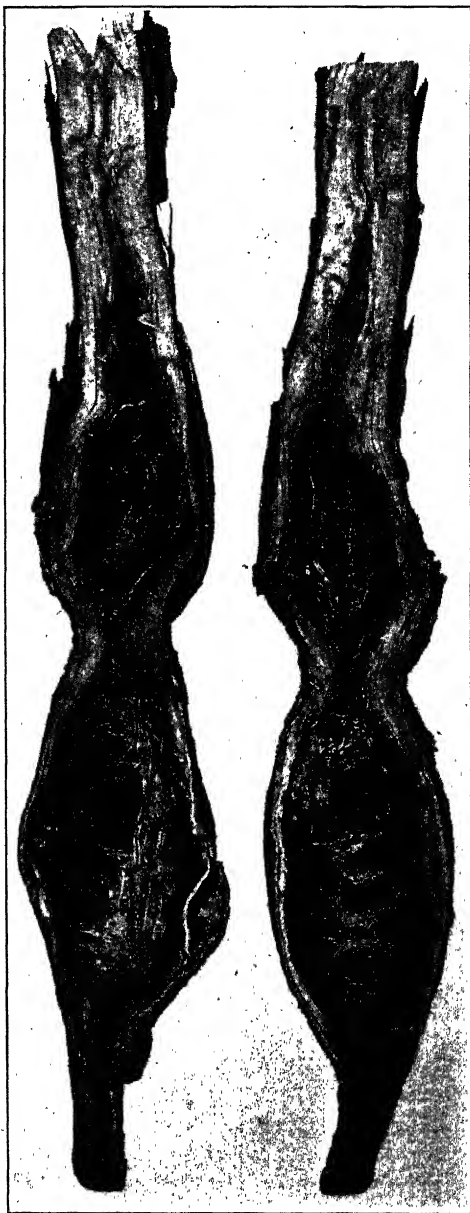


FIGURE 7.—Dried root of *Euphorbia intisy*, showing transverse plates formed by the shrinking of the aquiferous tissue. $\times \frac{1}{2}$

Although the manner of development of the hydriarhizas seems fairly clear, the actual mechanics involved is not at all understood and apparently it has not been investigated by any worker. Why during wet periods water should move with such disruptive force into these, and only these, portions of the root, and what it is that in the dry season effectively guards against too rapid water loss due to the pull exerted by the completely air-dry soil around the roots and the transpiration demands of the stem above, are questions which offer an interesting field of study for the physiologist.

Though these characteristic water-storage organs are able to withstand a tremendous amount of drought without injury, any rupture that exposes the aquiferous tissue to microorganisms is promptly followed by disintegration. Usually the entire hydriarhiza affected rots away, and frequently the decay extends along the stem, killing the entire plant; occasionally, however, only the aquiferous tissue disappears and a new hydriarhiza forms inside the hollow shell of the old one.

These peculiar hydriarhizas, which may reach a diameter of 15 cm. or even more, constitute an extremely valuable water reservoir. They undoubtedly are the chief factor that enables *intisy*, a plant that stores practically no water in the stem, to withstand the extremely arid conditions of southern Madagascar, where usually a 6-month period without rain occurs annually and where droughts lasting as many years are not unknown (9).

SUMMARY

Euphorbia intisy, a valuable rubber-producing plant, native in southern Madagascar, and first brought to the United States by the writer in 1928, is an upright, woody, almost leafless shrub or tree, reported to attain a maximum height of 7 meters. Its stem structure is characterized by early lignification, delayed phellogen formation, and the presence of very long, ramified latex cells that yield one of the best rubbers known. Certain irregularities of nodal anatomy are noted, one of which may possibly be of specific importance. The root system is unique. In addition to absorbing roots, which display six primary bundles, pericyclic cork, and other common characteristics of the roots of the genus *Euphorbia*, all of the main roots display alternate series of ordinarily thickened root segments and greatly swollen water-storage segments, here given the name hydriarhizas. These result from an enormous water intake by the cells of the pith-like axial tissue, certain of the primary bundles, the primary and secondary xylem rays, and the xylem parenchyma. Such storage of water enables the plant to withstand the extremely arid conditions of southern Madagascar.

LITERATURE CITED

- (1) COSTANTIN, J., and GALLAUD, I.
1905. NOTE SUR QUELQUES EUPHORBES NOUVELLES OU PEU CONNUES DE LA RÉGION SUD-OUEST DE MADAGASCAR, RAPPORTÉES PAR M. GEAY. Bul. Mus. Hist. Nat. Paris 11: 345-354.
- (2) ——— and GALLAUD, I.
1906. QUELQUES PLANTES À LATEX DE MADAGASCAR. Bul. Econ. Madagascar 6: 230-235.
- (3) DENIS, M.
1921. LES EUPHORBIÉES DES ILES AUSTRALES D'AFRIQUE. 151 p., illus. Paris. (Thèse). (Also published in Rev. Gén. Bot. 34: 5-64, 96-123, 214-236, 287-299, 345-353, illus. 1922.)
- (4) DRAKE DEL CASTILLO, E.
1900. NOTE SUR L'INTISY DE MADAGASCAR. Bul. Mus. Hist. Nat. Paris 6: 257-260, illus.
- (5) DUBARD, M., and VIGUIER, R.
1905. LE SYSTÈME RADICULAIRE DE L'EUPHORBIA INTISY. Rev. Gén. Bot. 17: [260]-271, illus.
- (6) FRON, G.
1900. NOTE SUR L'EUPHORBIA INTISY. Jour. Bot. [Paris] 14: [157]-163, illus.
- (7) GAUCHER, L.
1902. RECHERCHES ANATOMIQUES SUR LES EUPHORBIACÉES. Ann. Sci. Nat., Bot. (8) 15: [161]-309, illus.
- (8) JUMELLE, H.
1898. LES PLANTES À CAOUTCHOUC ET À GUTTA DANS LES COLONIES FRANÇAISES. 186 p., illus. Paris.
- (9) POISSON, H.
1909. NOTE SUR LES PLANTES À CAOUTCHOUC & À LATEX DU SUD ET DU SUD-EST DE MADAGASCAR. Rev. Gén. Bot. 21: [8]-31, illus.
- (10) PRUDHOMME, E.
1899-1900. LE CAOUTCHOUC SUR LA CÔTE EST DE MADAGASCAR. Rev. Madagascar 1: [366]-384, illus., 1899; 2: [43]-52, 1900.



RÔLE OF CHLORINE IN NUTRITION AND GROWTH OF THE TOBACCO PLANT AND ITS EFFECT ON THE QUALITY OF THE CURED LEAF¹

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INTRODUCTION

The effect on crop yields of applying to the soil common salt, or sodium chloride, and other chlorides has been a subject of considerable experimentation over many years. Numerous experiments likewise have been made with pot and water cultures to determine whether chlorine is an indispensable nutrient for higher plants and to learn more of its supposed action in stimulating growth. In general, the results have been so distinctly contradictory that no agreement has been reached as to whether chlorine is an essential element. It appears, however, that if it be necessary for normal growth and development the quantity required must be quite small. On the other hand, it is undoubtedly true that under suitable conditions the growth of some plants may be materially stimulated by the presence of proper quantities of chlorine in the soil or other culture medium. It seems that different plants vary considerably in their response to this element. The fairly extensive literature which has been developed on the effects of chlorine on plant growth generally has been reviewed by Tottingham (34)² and more recently by Lomanitz (22), and it is not necessary for present purposes to go further into the subject.

Until quite recently interest in the effects of chlorine on tobacco has centered almost exclusively on its relation to the combustibility of the cured leaf, little or no consideration being given to effects on growth of the plant. The object of this paper is to present data bearing on the effect of chlorine in stimulating growth in tobacco, its rôle in influencing water relations and nutritional processes in the plant, and its relation to color, combustibility, and other characteristics of the cured leaf.

EFFECTS OF CHLORINE ON YIELD OF TOBACCO

Prior to the beginning of the experiments to be considered herein, about the only data tending to establish a significant effect of chlorine on the yield of tobacco were furnished by fertilizer tests conducted over a period of years at the southwestern test farm of the Ohio Agricultural Experiment Station, located at Germantown (33). The soil is designated as Miami clay loam. In a 3-year rotation of tobacco,

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² Reference is made by number (italic) to "Literature cited," p. 646.

wheat, and clover, with a fertilizer applied to the tobacco crop which furnished 38 pounds of nitrogen, 30 pounds of phosphorus, and 75 pounds of potassium per acre, comparison is made of sulphate, nitrate, and muriate of potash. For the first 12 years (1903-1914) the average yields of tobacco produced with the three forms of potash in the order mentioned were, respectively, 1,213, 1,204, and 1,352 pounds per acre. Thus the muriate showed a gain of about 145 pounds over the sulphate and nitrate. Where lime was applied in addition to the fertilizer, the respective yields with sulphate and muriate were 1,175 and 1,200 pounds. For the succeeding period of 9 years (1915-1923) the average yields obtained with the sulphate, nitrate, and muriate were 1,025, 1,040, and 1,069 pounds, respectively. On the limed plots the sulphate produced 957 pounds and the muriate 947 pounds. Obviously the initial increases obtained with the muriate have not been maintained. Moreover, the initial gain in yield from the muriate is not so pronounced where lime was applied to the soil.

In connection with an extensive series of fertilizer tests with flue-cured tobacco, conducted on the Durham sandy loam and coarse sandy loam soil types at the Tobacco Branch Station of the North Carolina Department of Agriculture located at Oxford, provision was made for field study of the comparative values of different forms of potash, with special reference to the sulphate and the muriate. It subsequently developed that the magnesium supply is an important limiting factor in crop yields on the soils in question, and it became necessary to make provision for this element in the study of the potash salts. Similar tests with potash salts were carried out for a time on Durham sandy loam soil at Reidsville, N. C.

In the first series at Oxford, beginning with 1913, comparison was made with the sulphate and muriate of potash at rates to supply 80 pounds of potash per acre in conjunction with 40 pounds of ammonia derived from dried blood and 64 pounds of phosphoric acid derived from superphosphate. The plots were run in duplicate and were located on three separate fields, each of which was run in a 3-year rotation of tobacco, oats, and corn prior to 1919; subsequently rye harvested for grain was substituted for the corn crop. The oats received 100 pounds of nitrate of soda per acre as fertilizer; the corn was fertilized with 100 pounds each of nitrate of soda and cottonseed meal and 200 pounds of superphosphate; the rye was not fertilized. Beginning with 1920, one-half of each plot received dolomitic limestone broadcast at the rate of 1 ton per acre every third year, prior to transplanting tobacco. The soil type was Durham coarse sandy loam.

On the same soil type a second series of tests was run with the sulphate and muriate salts applied at rates to supply 36 pounds of potash per acre in conjunction with the same supply of ammonia and phosphoric acid as in the first series. Two different sources of muriate and sulphate, namely, domestic and imported, were used. Tobacco was grown each year on the same plots, and the treatments were not duplicated. However, the tests were run in three sections, namely, without addition of limestone, and with 1,000 pounds each of dolomitic and calcitic limestone applied in the drill each year.

These tests also were conducted for a shorter period on Durham sandy loam soil at Reidsville.

The third series at Oxford involves a comparison of sulphate and muriate applied at rates to supply 12, 24, 36, and 80 pounds of potash

per acre. The supply of ammonia and phosphoric acid was the same as in the first series, except that a dicalcic phosphate was substituted for superphosphate. For the first three years the treatments were duplicated, but thereafter one plot of each treatment received every third year one ton of dolomitic limestone. Tobacco was grown on the same plots every year, and the soil was Durham sandy loam. This series also was duplicated on the same soil type at Reidsville, the only difference in treatment being that superphosphate was used in place of dicalcic phosphate.

The detailed results of the above-described tests have been presented in large part elsewhere (25), though not with special reference to the effects of chlorine. It will suffice for present purposes to present only the average results of these tests, which have extended over periods ranging from 3 to 14 years. The data are shown in Table 1.

TABLE 1.—*Effect of chlorine on yield and value of tobacco, as indicated by comparative results with equivalent quantities of high-grade sulphate and muriate of potash, with and without application of limestone*

Locality, soil type, series, and treatment No.	Duration of tests	Kind of lime applied	Potash applied per acre	Yield of tobacco per acre with potash supplied as—		Price per 100 pounds of tobacco when potash was supplied as—	
				Sulphate	Muriate	Sulphate	Muriate
Oxford, N. C.; Durham coarse sandy loam:							
Series 1—	Years		Pounds	Pounds	Pounds	Dollars	Dollars
1, 2	14	None	80	754	852	18.50	21.40
3, 4	7	Dolomite	80	972	1,036	20.35	21.68
Series 2:							
1, 2	6	None	36	587	704	24.50	24.61
3, 4	6	Calcite	36	630	644	22.40	24.01
5, 6	6	Dolomite	36	848	929	27.85	29.40
Reidsville, N. C.; Durham sandy loam:							
Series 1—							
1, 2	3	None	36	921	914	15.53	19.34
3, 4	3	Calcite	36	932	1,049	17.95	18.42
5, 6	3	Dolomite	36	937	1,006	18.92	18.72
Oxford; Durham sandy loam:							
Series 3—							
1, 2	10	None	12	491	589	25.65	27.72
3, 4	10	do.	24	599	668	26.45	30.56
5, 6	10	do.	36	593	698	27.07	30.89
7, 8	10	do.	80	643	698	25.79	29.24
Series 4—							
1	7	Dolomite	12	674	687	23.18	25.30
2	7	do.	24	695	814	23.80	25.96
3	7	do.	36	807	839	26.10	24.74
4	7	do.	80	652	796	24.78	26.34
Reidsville; Durham sandy loam:							
Series 2—							
1	5	None	12	715	730	15.82	21.16
2	5	do.	24	690	760	18.00	19.78
3	5	do.	36	786	832	19.05	22.44
4	5	do.	80	768	898	18.66	21.90

The results as a whole leave no doubt of the fact that under the conditions muriate of potash tends to give an appreciable increase in yield as compared with the sulphate. Taking into account the number of years covered by the several tests, the replications, and the variations in the treatments, there are in all 193 comparisons of sulphate and muriate, and the average yields under all conditions are 734 and 807 pounds, respectively. The muriate thus shows an average increase over the sulphate of 73 pounds, or 10 per cent. It is not to be expected, of course, that the muriate will show an advantage

over the sulphate on all soils or under all conditions. The results in Table 1 show a similarity to those reported in Ohio, in that addition of limestone to the soil tends to reduce or eliminate the differences in yield between the two forms of potash. There are instances in the table also in which little or no difference in yield is to be seen, even where no lime was used. However, experiments in other localities, together with numerous observations on the crops of growers, indicate that the response in growth of the tobacco plant resulting from application of chlorides is rather general, especially on light soils having a low water-holding capacity. That the increased yield obtainable with muriate of potash is to be attributed to the effects of the chlorine ion is indicated by the facts that sodium chloride also has been found to give a decided increase in growth on the Oxford soils, and, as is later shown, potash applied in the form of sulphate is readily absorbed from these soils by the plant. Moreover, the stimulating effect of chlorine also is seen when ammonium chloride is used in comparison with other sources of fertilizer nitrogen.

The quantity of chlorine required to obtain maximum yields compatible with high quality of product is naturally a matter of practical importance. The effect of a given quantity varies according to the conditions, so that the quantity required for the best results can only be roughly approximated. The data supplied by series 1 and 2 of the tests at Oxford and series 1 of those at Reidsville merely show that at certain given rates of application muriate of potash in most instances gives a larger yield than an equivalent quantity of the sulphate. The results with muriate applied at different rates in series 3 and 4 at Oxford and series 2 at Reidsville, however, indicate on the whole that under the conditions of the tests maximum yields may be attained with the rates supplying 24 to 36 pounds of potash per acre. In the grades of muriate used the content of chlorine ordinarily is only slightly less than the content of potash, so that apparently maximum results are to be obtained with approximately 20 to 30 pounds of chlorine per acre. The parallel results with the sulphate do not indicate that potash was a serious limiting factor at the rates in question. It may be tentatively concluded, therefore, that on light soils of the type here involved the stimulating effects of chlorine on growth may be largely attained by applying about 25 pounds per acre of this element.

RELATIVE AVAILABILITY OF POTASH IN MURIATE AND SULPHATE AND COMPARATIVE READINESS WITH WHICH THE CHLORIDE, SULPHATE, AND PHOSPHATE IONS ARE ABSORBED BY THE PLANT

The increased yields of tobacco obtained with muriate of potash in comparison with the sulphate naturally raise the question whether the potash applied to the soil in the latter form is as readily absorbed by the plant as that applied as muriate. To answer this question, the potash content of the tobacco grown in certain years in some of the previously described tests in series 1 and 3 at Oxford was determined by analysis. Similar data were also obtained on tobacco that received potash in the form of sulphate in series 1 at two rates not previously mentioned, namely, 40 and 160 pounds per acre. The analyses were made to include the effects of the sulphate and muriate on the absorption by the plant of calcium, magnesium, sulphur, and chlorine. The results are shown in Table 2.

TABLE 2.—*Comparative effects of high-grade sulphate and muriate of potash applied as fertilizer at different rates on the content of potash, lime, magnesium, sulphur, and chlorine in tobacco grown on sandy loam soil at Oxford, N. C.*

Series and treatment No.	Potash applied		Part of plant analyzed	Mineral constituents of tobacco					
	Form	Quantity per acre		K ₂ O	CaO	MgO	Total sulphur (as SO ₃)	Sulphate sulphur (SO ₃)	Cl
Series 1:		Pounds		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1.....	Sulphate.....	80	Leaf.....	3.59	3.29	0.49	1.65	1.03	0.39
2.....	Muriate.....	80	do.....	3.45	3.39	.58	.88	.35	3.68
5.....	Sulphate.....	40	do.....	3.05	1.55	1.08	.36
6.....	do.....	160	do.....	4.96	2.67	.44	2.00	1.38	.80
Series 3:									
3.....	Muriate.....	12	do.....	1.38
4.....	Sulphate.....	12	do.....	1.28
5.....	do.....	36	do.....	2.78	4.84	.33	.95	.58	.44
6.....	Muriate.....	36	do.....	2.22	5.14	.45	.63	.28	1.95
7.....	Sulphate.....	80	do.....	3.36	1.13	.65	.83
8.....	Muriate.....	80	do.....	3.1155	.15	3.19
5.....	Sulphate.....	36	Stalk.....	1.90	1.18	.23	.48	.28	.45
6.....	Muriate.....	36	do.....	2.00	1.15	.18	.35	.15	1.00
7.....	Sulphate.....	80	do.....	3.3295	.63	1.67
8.....	Muriate.....	80	do.....	3.4648	.20	3.40

It will be seen at once that there is no substantial difference in the potash content of the tobacco where the two potash salts are used in equivalent quantities. The sulphate tends to give a slightly higher percentage of potash in the leaf, but this is fully offset by the fact that a larger yield of leaf is produced by the muriate. Evidently the differences in yield are not due to difference in availability of the potash supplied by the two salts. It is seen, also, that with both salts increase in quantity of potash added to the soil results in increased content of potash in the tobacco.

Increase in quantity of the two potash salts applied to the soil resulted in progressive decrease in calcium content of the tobacco, but there was no decided difference between the action of the two salts in this respect. The situation is different, however, in the case of magnesium. The Oxford soils are markedly deficient in magnesium, and in all cases the content of this element in the tobacco is low. Increasing quantities of sulphate of potash did not greatly affect the absorption of magnesium, but in each instance the muriate in comparison with the sulphate enabled the plant to absorb a greater supply of magnesium. It was, in fact, the striking difference in effects of sulphate and muriate on visible symptoms of sand drown (magnesium hunger) in tobacco in these tests which first directed attention to the prevalence of magnesium-deficiency symptoms in tobacco grown on light sandy and sandy loam soils. In a study of the effects of different fertilizer treatments on the composition of tobacco in connection with the plot tests at Germantown, Ohio, previously mentioned, Ames and Boltz (1) likewise found a definite increase in magnesium content of tobacco when muriate of potash replaced sulphate or nitrate of potash in the fertilizer, although in this case the soil evidently was reasonably well supplied with magnesium. It seems, therefore, that muriate of potash increases the solubility of the magnesium present in the soil, but obviously when the total magnesium supply of the soil is quite low the solvent action of the chloride can hardly be expected to persist over many years. In the case of the Oxford soils it has been found, in fact, that after a few years'

continued use muriate of potash ceases to be effective in preventing symptoms of magnesium deficiency.

It seems reasonably clear that, on some soils at least, the increased yield obtained with muriate in comparison with other forms of potash is due in part to the solvent action of the muriate on the magnesium supply of the soil, thus enabling the plant to obtain more nearly its minimum magnesium requirements. This would be in accord with the observations of Ames and Boltz (1) and more recently those of Anderson and Swanback (2) that applying magnesium limestone to the soil increases the magnesium content of tobacco at the expense of calcium and potassium. Thus a possible explanation is found of the fact that the muriate of potash is likely to be less effective in increasing yield when magnesium limestone has been applied to the soil, for both treatments tend to accomplish the same result.

Turning to the absorption of the sulphate and chloride ions by the plant, the data in Table 2 show that in all cases the sulphate of potash increases the content of total sulphur and sulphate sulphur in the tobacco, while the muriate increases the chlorine content. However, the gains in sulphur compounds are relatively small, while the increases in chlorine content are quite large. These results are supported by numerous observations of investigators on other plants. Thus Hoagland and Davis (19) found that even in water cultures the chloride ion is much more readily absorbed than the sulphate ion. In the tests with tobacco under consideration it will be observed that when the muriate is used the tobacco contains approximately as much chlorine as potash, whereas the use of the sulphate in equivalent quantities results in a much lower sulphur content. These facts afford additional evidence that the stimulating action of chlorides on plant growth is due, at least in part, to actual absorption of chloride ions.

That the chloride ion is absorbed by tobacco with great ease has been confirmed by a number of additional tests. In one instance tobacco seedlings that had been liberally fertilized with a low-grade muriate of potash were found to contain more than 10 per cent chlorine. In the experiments under discussion the rate of application of phosphoric acid was uniform, but numerous published data show that the capacity of the tobacco plant to absorb the phosphate ion is quite limited and that the phosphoric-acid content of the leaf rarely exceeds 1 per cent. Anderson, Morgan, and Nelson (3) report that with fertilizer applications of phosphoric acid ranging from 53 to 250 pounds per acre the range in phosphoric acid in the cured leaf was only 0.61 to 0.65 per cent for the light grade and 0.70 to 0.83 per cent for the dark grade. Ames and Boltz (1) likewise found that the use of 480 pounds of superphosphate per acre had practically no effect on the phosphoric-acid content of the leaf.

RELATION OF CHLORINE TO DROUGHT RESISTANCE IN TOBACCO

In the tests with sulphate and muriate of potash at Oxford, N. C., independently of characteristic symptoms of magnesium deficiency, certain clearly defined differences in growth and appearance of the plants fertilized with the two salts could be seen. Where the sulphate was used the leaves of the plants showed a relatively dark green color and to the touch seemed to be comparatively thick. The leaves were relatively small in size. Where the muriate salt was used the leaves

possessed a bright, rather light green color and were thinner and somewhat larger in size. As the plants approached maturity there was at times a tendency toward physiological breakdown in the leaf where the sulphate had been used in the fertilizer. This breakdown appeared in the form of numerous dead spots and blisters, and occasionally also the margins of the leaves withered and died. These symptoms were more pronounced in dry weather, especially when the dry weather followed a period of wet weather. There was little or no evidence of this breakdown when muriate of potash was substituted for the sulphate. The physiological breakdown that has been observed in the leaves of the plants on the sulphate plots appears to be identical with the disease that Fromme and Wingard (14) have designated as "drought spot."

A striking instance of the serious proportions which this drought effect may assume and of the definite protective action afforded by chlorine was seen in the tobacco crop produced in the vicinity of Oxford, N. C., in 1925. After the crop had been set in the field and had made considerable growth a severe drought prevailed for some weeks. In many instances the margins of the lower leaves of the plants dried, turned brown, and eventually dropped off. After rain had fallen, subsequent growth of the crop was normal. The soils commonly employed in growing the type of tobacco in question are of a light, sandy character and have a low water-holding capacity, so that in the absence of occasional showers they are likely to become hot and quite dry. In these circumstances the tobacco plant naturally experiences difficulty in maintaining its water supply.

Upon the conditions that prevailed in 1925 striking differences were observed between the experimental plots at Oxford that were fertilized with sulphate and with muriate of potash, respectively. All of the data in Table 2 for series 1 and those for treatments 7 and 8 of series 3 were obtained from material grown under the above-mentioned conditions. The burning of the margins of the leaf, popularly spoken of as "rim fire," was quite severe on all the sulphate treatments in both series except where the potash application was 160 pounds per acre. Except in the lowest rate of application, the muriate treatments prevented any material injury from burning. Potash as such failed to protect the leaves from burning when present in the tissues to the extent of 3.5 per cent of the dry weight and accompanied by a chlorine content of less than 1 per cent. When the chlorine content was increased to 3 per cent or more, evidence of injury was practically absent, and with a chlorine content of about 2 per cent (treatment 6, series 3) there was only slight damage. Again, with a chlorine content of about 0.8 per cent but with an increase in potash content to 5 per cent (treatment 6, series 1) there was effective protection. It appears that both potash and chlorine exercise a protective action against drought, but the latter seems to be decidedly more effective than the former.

The protective action of muriate of potash as compared with the sulphate against injury from drought has been observed in a number of instances in the crops of growers. Several cases were seen in the vicinity of Oxford in 1925. Where the fertilizer did not contain significant quantities of chlorine, the margins of the leaves died off and numerous blisters or spots developed between the larger veins. Similar observations were made in different localities in 1928 where

exceptionally heavy rains were followed by a period of hot sunshine. It must be recognized that injury of the sort in question may subsequently result as a sequel to heavy rainfall or prolonged wet weather as well as directly from prolonged drought.

If it be true that chlorine in the tissues enables the leaf to resist injurious or fatal drying under certain conditions, it should be possible to show that the water content of the leaf is measurably increased under these conditions. Considerable data tending to demonstrate that this is true have been obtained at Upper Marlboro, Md. In 1926 a series of observations was made through the growing season on the water content of the leaves of plants when equivalent quantities of both potash and magnesia were applied in the form of sulphate and chloride, respectively. Similar observations also were made on the leaves of plants fertilized with ammonium sulphate and ammonium chloride, respectively, at rates to supply a fixed quantity of nitrogen. In the first case 64 pounds of phosphoric acid per acre, and in the second case 60 pounds of this nutrient, were included in the fertilizer in the form of dicalcic phosphate. The tests with the potash salts were located on Collington loamy sand, and those with the ammonia salts on Collington fine sandy loam. The first samples of leaf were taken on July 26, 18 days before the plants in the tests with sulphate and muriate of potash were topped. In the other series of tests the plants were not topped till the first blossom opened, which occurred August 26. On each plot the moisture content of the soil to a depth of 6 inches was determined each time the leaf samples were taken. The results of the observations are shown in Table 3.

TABLE 3.—*Effects of chlorine in fertilizer on water content of tobacco leaves during the growing season at Upper Marlboro, Md.*

Plot No.	Nitrogen in fertilizer		Potash in fertilizer		Quantity of chlorine in fertilizer per acre	Water content of leaves						
	Form	Quantity per acre	Form	Quantity per acre		July 26	Aug. 3	Aug. 10	Aug. 26	Sept. 2	Sept. 9	Average
						Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
19	Ammonium sulphate.	80	Sulphate.	40	0	81.68	85.66	82.20	84.23	83.77	83.49	83.50
20	Ammonium chloride.	80	do.	40	202	85.35	88.88	84.38	86.73	85.99	84.40	85.96
10	Dried blood.	40	Muriate.	80	130	86.14	89.37	83.04	85.16	81.16	80.19	84.18
11	do.	40	Sulphate.	80	0	82.76	85.76	79.49	82.39	77.32	78.78	81.08
Water content of soil												
19	Ammonium sulphate.	80	do.	40	0	7.51	6.78	4.70	10.80	-----	9.27	7.81
20	Ammonium chloride.	80	do.	40	202	7.63	6.76	4.92	11.19	-----	9.05	7.91
10	Dried blood.	40	Muriate.	80	130	3.52	3.55	2.14	8.21	3.72	6.38	4.59
11	do.	40	Sulphate.	80	0	3.52	3.54	2.04	7.91	4.26	6.11	4.56

* Plot 10 also received 40 pounds MgO per acre in the form of chloride, and plot 11 received a like quantity of MgO in the form of sulphate.

It will be observed that in both cases the addition of 100 to 200 pounds of chlorine per acre consistently maintained a rather marked increase in water content in the leaf throughout the growing season.

These results, which have been confirmed by a number of additional observations, show that chlorine does enable the tobacco leaf to resist desiccation, and this effect might easily account for the increase in yield of the tobacco crop often obtained when the fertilizer contains a considerable percentage of chlorine. There is considerable difference in the water content of the two soil types used in the tests, and there seems to be a relation between this difference in the moisture content of the soil and the water content of the leaf. Also, variation in soil moisture through the season appears to cause considerable variation in water content of the leaf. Finally, there appears to be on the whole a downward trend in water content of the leaves as they grow older. However, the distinctive effect of the chlorine is always in evidence.

EFFECT OF CHLORINE ON CARBOHYDRATE METABOLISM IN THE PLANT

In the preceding paragraphs it has been shown that the presence of chlorine in the soil may enable the plant to absorb more readily the necessary quantity of magnesium to meet nutrition requirements, while the presence of chlorine in the tissues of the leaf enables it to resist injurious desiccation. Thus, the growth of the plant may be stimulated and the yield of leaf may be increased. It now remains to show that a too liberal supply of chlorine in the soil may produce decided injury to the plant, apparently not by direct toxic action but by serious interference with carbohydrate metabolism.

Here, again, it seems that the water relations of the plant are involved. The influence of the water content on carbohydrate metabolism in the plant was first demonstrated in 1914. In that year, in connection with studies on tobacco curing, it was shown in the Office of Tobacco and Plant Nutrition (16) that partial wilting of the green leaf has a decided effect in stimulating the enzymatic processes involved in conversion of starch into reducing sugars. In the same year Lundegårdh (23) also found that under favorable conditions decrease in water content of plant tissues increase the transformation of starch into sugars, and, conversely, increases in water content causes re-formation of starch. These reactions have since been studied in some detail by Schroeder and his associates (32), including Bruns (7), and have been shown to have wide application. It was found that in the presence of starch, saccharose is formed as the water content decreases, so that the content of saccharose in the tissues is a function of the water content. This relation holds true in light and in darkness. In darkness the hexose content of the plant tissues also increases with decrease in water content. It seems clear that these transformations in relation to water content are of fundamental importance in the nutrition processes of the plant.

When the quantity of chlorine applied to the soil is in excess of 100 pounds per acre, and in some cases when the quantity used is as low as 40 to 60 pounds per acre, instead of the generally beneficial effects obtained with lighter applications the appearance and development of the leaf are commonly modified to a striking degree. The leaf surface has a sleek, exceptionally smooth or glabrous appearance. To one familiar with this effect of chlorine the appearance of the leaf is quite distinctive and easily recognized. In extreme cases the margins of the leaves curve upward in a characteristic fashion. (Fig. 1.)

Perhaps the most striking characteristic of such leaves is their extraordinary thickness and brittleness. The contrast in thickness between normal leaves and those showing the typical chlorine effect can be seen in Figure 2. The thickened leaf is from a plant that received a liberal application of chlorine in the form of calcium chloride. As seen in cross section, the thickness is several times that of the normal leaf. The leaf cells become gorged with starch, and the tissues are quite brittle. In all the cases studied these evidences of malnutrition have been associated with a high content of chlorine in the tissues, namely, 4 to 7 per cent or more. The malnutrition symptoms have been obtained in pot tests as well as in the field, and with the chlorides of potassium, sodium, and calcium, ammonium chloride, and hydrochloric acid.

When the available supply of chlorine is sufficient to produce the above-described nutritional disturbances to a marked degree, the



FIGURE 1.—Tobacco plant in the field, showing marked curling of the leaves as a result of absorption of an excessive quantity of chlorine from the fertilizer used

growth of the plant may be checked. A number of such instances have been observed in the field, and in some cases applications to plant beds of fertilizers containing high percentages of chlorine have resulted in the death of the tobacco seedlings. It appears that the character of the soil and the weather conditions have a material effect on the quantity of chlorine required to produce significant injury to the tobacco crop in the field. On some soils applications of as much as 75 pounds per acre of chlorine have failed to produce notable injury, while, as already indicated, on the other soils material damage has resulted from considerably lower rates of application. Apparently, chlorine injury in the field is most likely to occur on light sandy soils which have

only slight buffering properties. That weather conditions also greatly affect the results, even on the same soil, is shown in the following paragraphs.

To obtain information as to the effect of chlorine on the chemical composition of leaf in association with the extreme thickening, partial analyses were made of green leaves collected from plants receiving treatments 10 and 11 at Upper Marlboro, Md., the details of which already have been given. As indicated in Table 3, plot 10 received about 130 pounds of chlorine per acre. In 1923 the leaves of the plants on this plot showed the characteristic effect of a high chlorine content, while in 1924 these effects were not pronounced, apparently because much of the chlorine added in the fertilizer was lost to the plant as a result of leaching. The fresh leaves collected in 1923 and 1924 were quickly weighed and the areas then traced on paper. After removing the midribs, the half leaves remaining were exposed to

chloroform vapors to prevent further changes in composition and then dried at air temperature. The organic acids were determined by the method of Kissling, while the methods of the Association of Official Agricultural Chemists were employed for the other determinations. The results are summarized in Table 4.

TABLE 4.—*Effect of a high chlorine content in the plant tissues on the weight, water content, size, and chemical composition of tobacco leaves grown at Upper Marlboro, Md.*

Year and plot No.	Quantity of chlorine in fertilizer per acre	Total area of 10 leaves	Weight of 10 leaves		Weight of leaf per square foot		Weight of water per square foot of leaf	Water content of leaves	Dry material in leaves
			Fresh	Dry	Fresh	Dry			
	<i>Pounds</i>	<i>Sq. feet</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Per ct.</i>	<i>Per ct.</i>
1923 {10.....	130	14.63	524.83	82.56	35.874	5.643	30.231	84.27	15.73
1923 {11.....	0	14.58	363.58	68.62	24.937	4.706	20.231	81.13	18.87
1924 {10.....	130	10.00	367.67	65.50	36.767	6.550	30.217	82.19	17.81
1924 {11.....	0	8.99	323.00	60.70	35.929	6.752	29.177	81.21	18.79

Year and plot No.	Composition of dry matter in leaves							Weight of constituents in 10 square feet dry leaf					
	Chlorine	Starch	Sucrose	Reducing sugars (as invert)	Organic acids	Protein	Pure ash	Starch	Sucrose	Reducing sugars (as invert)	Organic acids	Protein	Pure ash
	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>
1923 {10.....	4.80	33.51	0.03	3.93	1.58	10.12	-----	13.91	0.02	2.22	0.89	5.71	-----
1923 {11.....	.22	24.98	.14	4.31	3.96	11.12	-----	11.76	.07	2.03	1.86	5.23	-----
1924 {10.....	1.18	23.74	.00	7.13	3.47	11.50	7.59	13.82	.00	4.67	2.27	7.63	4.97
1924 {11.....	.10	27.53	.04	6.89	4.99	11.44	7.61	13.59	.03	4.65	3.37	7.72	5.14

The fact that on plot 10, which received more than 100 pounds of chlorine per acre, the leaves of the plants failed to show in 1924 the striking peculiarities in appearance and abnormal thickening observed in 1923 is readily explained by the decided difference in chlorine content of the leaves in the two seasons. In 1924 the leaves contained only a little more than 1 per cent of chlorine, whereas in 1923, with the same quantity of chlorine added to the soil, the content of the leaves was fully four times as high. The effects of the chlorine in the two years were quite different. With slightly more than 1 per cent of this element in the leaf tissues in 1924, the effect was to increase considerably the area of the leaf, while per unit of area the water content, the fresh weight, and the dry weight were only slightly changed. In other words, the increase in total fresh weight and in dry weight of the leaf was due almost entirely to increase in the total area and not to increase in thickness of the leaf. With an increase in chlorine content of the leaves to nearly 5 per cent in 1923 there was no material effect on total leaf area, but the water content and the fresh weight per unit of area were greatly increased and the dry weight also was materially increased. Manifestly, the green leaf was much thickened. Measurements on the cured leaf of the 1923 crop showed an increase of one-third in thickness of leaf from plot 10 over that of plot 11.

With respect to chemical composition, about the only clearly evident effect of the chlorine in 1924 was to reduce the content of organic acids in the leaf, an effect which will be discussed in later paragraphs. In 1923, however, in association with the more decided action of the chlorine in maintaining a high water content, there was a marked accumulation of starch in the tissues of the leaf. Observation has shown that this result is typical, and it seems reasonable to suppose that it is but a special case of the general principle that a high water content in the leaf tissues tends to bring about abnormal accumulation of starch. It appears that carbohydrate metabolism is checked and growth of the plant may be practically stopped. It is evident that amylolytic activity is in some way adversely affected. The cause of the accelerated transformation of starch in wilting leaves has been studied by Bruns (?), but this problem has not yet been fully solved.

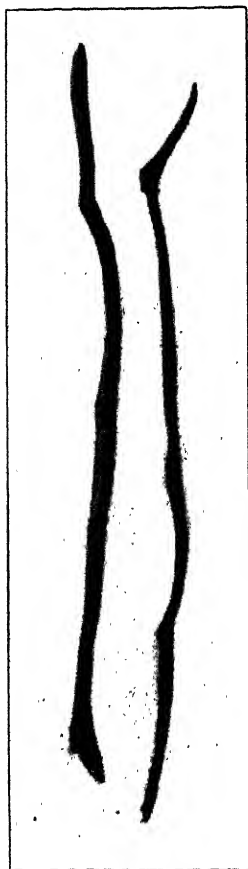


FIGURE 2.—Cross sections of green tobacco leaves, showing marked thickening of the leaf (left) resulting from heavy applications of chlorine to the soil, as compared with the normal leaf (right). The thickened leaves are highly turgid and extremely brittle

To ascertain whether the interference with amylolytic activity resulting from the presence of chlorine in the leaf is directly dependent on a high water content or is of a more permanent nature, observations were made on the comparative rates of starch transformation during the curing process in tobacco leaves taken from field plots 19 and 20 in one series and plots 10 and 11 in another series at Upper Marlboro, Md. The fertilizer treatments of these plots are shown in Table 3. It is well known that rapid depletion of accumulated starch in the leaf is a characteristic feature of curing under normal conditions, and, of course, the harvested leaves also quickly lose a part of their content of water. Immediately after harvesting one-half of each leaf was cut off along the midrib and dropped into boiling alcohol to arrest metabolic processes. The remaining portions of the leaves with midribs attached were placed in a ventilated dark chamber in which a relative humidity of about 82 per cent and a temperature of 80 to 85° F. were maintained. After 68 hours the midrib was removed from each half leaf and the re-

maining material was dropped into boiling alcohol. Starch and sugars were determined in each sample according to the methods previously mentioned. The results, calculated on a basis of the original fresh weight of the leaves in each case, are shown in Table 5.

TABLE 5.—*Effect of chlorine applied in the fertilizer on starch digestion in tobacco leaves during a curing period of 68 hours, Upper Marlboro, Md.*

Plot No.	Chlorine applied per acre	Leaf material analyzed	Composition of leaf, calculated on basis of original fresh material		
			Starch	Sucrose (as invert)	Reducing sugars (as invert)
	<i>Pounds</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
10.....	130	{Original.....	7.36	0.26	0.44
		{Partially cured.....	.94	.86	3.51
11.....	0	{Original.....	4.91	.18	.67
		{Partially cured.....	.49	.61	2.21
20.....	202	{Original.....	2.50	.19	.39
		{Partially cured.....	.31	.17	.66
19.....	0	{Original.....	1.44	.17	.70
		{Partially cured.....	.15	.13	.41

The addition of chlorine to the soil resulted in a decided increase in starch content in the fresh leaf. Partial loss of water by the leaves after harvest, however, was associated with rapid decrease in starch, the rate of decrease being approximately the same as in the leaves from plants that received no chlorine in the fertilizer. Independently of the chlorine factor, the content of sugar at the end of the curing period decreased progressively with decrease in the original content of starch. These results indicate that the presence of chlorine in the tissues does not appreciably interfere with the hydrolysis of starch or the utilization or transport of the products of hydrolysis which normally take place in association with loss of water in the curing process. In other words, the results are in agreement with what is to be expected if the effect of chlorine in causing abnormal accumulation of starch in the tissues of the plant is but a special case of the general tendency for an abnormally high water content in the tissues to check the dissolution and utilization of starch.

INFLUENCE OF CHLORINE ON QUALITY OF CURED LEAF

That the salt content of sea water impairs the free combustibility of tobacco was known to the early settlers in the tidewater region of Virginia. Clayton (9), writing in 1688, states:

Tobacco that grows on low Lands as far as the Salts, tho' the Plant be never overflowed with salt Water, yet the Ground that feeds the Plant being impregnated with salt Water, that Tobacco smoaks not pleasantly, and will scarcely keep Fire; but do all that a Man can, will oft go out, and gives much trouble in frequent lighting the Pipe * * * *

The first scientific study of the subject was undertaken by Schloesing in 1860 (31), who reached the conclusion that the degree of combustibility of the tobacco leaf is more or less proportional to the content of potash existing in the form of salts of malic and citric acids. Nessler (26, 27) found that chlorine in the leaf has an especially injurious effect on its combustibility, while potash tends to offset this unfavorable effect. Much of the subsequent discussion by numerous investigators relative to the combustibility of tobacco has centered around these two theories of Schloesing and Nessler. In reality the two theories are more closely related than might appear on the surface, and that both are correct when properly applied can no longer be doubted.

Without undertaking a detailed discussion of the subject, it will be sufficient to point out that the importance of one or the other or both

of these theories has been demonstrated or supported by the work of Kosutány (21), Fesca and Imai (12), Van Bemmelen (6), Mayer (24), Ogier (28), Patterson (29), Hissink (18), Garner (15), Wagner and his associates (35), Ames and Boltz (1), Frear and his associates (18), Haley, Nasset, and Olson (17), and Anderson, Nelson, and Swanback (4). Preissecker (30) has made more readily available Pezzolato's analysis of the problem of combustibility in tobacco, in which, also, the importance of both of these factors is recognized. While not in all cases minimizing the significance of chlorine and organic salts of potash, Johnson (20), Barth (5), Cserhádi (11), Cohen (10), and Carpenter and Allen (8) have suggested that their effects may be masked by other factors which play a part in combustibility. It appears unnecessary to present the extensive data that have been obtained in recent years in the Office of Tobacco and Plant Nutrition further demonstrating the injurious effect of chlorine on the combustibility of tobacco. Some of the data have been summarized by Moss and his associates (25), and other portions are reserved for consideration in future publications. In fertilizer practice sound application of the principles here involved requires careful consideration of the type of tobacco and its uses in manufacture, the soil, cultural practices, and other pertinent factors. The requirements as to combustibility are most rigid in the case of cigar leaf but are also of considerable importance in all types used for cigarettes and for pipe smoking.

With a given content of potash in the leaf, the proportion of the total that may exist in the form of organic salts depends primarily on the relative content of potash and of chloride and sulphate radicals, although of course the content of lime and magnesia is of more or less moment. The significance of the chlorine-potash ratio, therefore, may be considered as due largely to its inherent bearing on the content of organic potash in the leaf. It has been shown in the present paper that the chloride ion is much more readily absorbed from the soil by the plant than the sulphate ion. The natural effect of applying the chlorides of potassium, sodium, calcium, or magnesium to the soil is a sharp increase in chlorine content of the plant, and the chlorine-potash ratio may be expected to be relatively high. On the other hand, when soluble sulphates are applied to the soil the sulphate ion is absorbed by the plant with difficulty. Moreover, the absorbed sulphate ions in part undergo transformation, entering into the make-up of certain proteins. It is clear that the potassium or other metal occurring in the plant in the form of chloride is not available for the formation of salts of organic acids except as replaced by other metals. In general, therefore, when muriate of potash is used as a fertilizer the chlorine-potash ratio in the tobacco leaf will be high, both the chloride and potassium ions being readily absorbed, while the content of potash salts of organic acids will be comparatively low. Apparently, the latter result explains in part the unfavorable effect of chlorine on the combustibility of tobacco. When sulphate of potash is used as a fertilizer the potassium ion is more readily absorbed than the sulphate ion, so that the sulphate-potash ratio in the leaf is low and a large proportion of the potash is free to combine with organic acids. To a considerable extent sulphate of potash as a fertilizer functions like the carbonate.

Just as in the case of the green plant in the field, chlorine has a decided effect on the water content of the cured tobacco leaf. It has

been frequently observed that when a fertilizer with a high chlorine content is used in growing the crop the cured tobacco leaf retains sufficient moisture for handling without breakage or damage under conditions where other comparable leaf is quite brittle and crumbles when handled. To obtain definite information on this effect of chlorine in the leaf, a number of samples of leaf tobacco grown with and without the addition of chlorine in the fertilizer were exposed to fixed conditions of temperature and relative humidity, and the water content of the samples was determined. The material used consisted of flue-cured leaf from treatments 1 and 2 of series 1 and treatments 7 and 8 of series 3, shown in Table 2; and Maryland leaf from plots 10, 11, 19, and 20, the treatments of which are indicated in Table 3. The tests with material from treatments 7 and 8 of series 3 in Table 2 covered two different years, and in addition to plots 19 and 20 of Table 3 a third plot, in which the nitrogen was derived from nitrate of soda, was included in selecting material. All samples were cured normally except those from plots 10 and 11 of Table 3. In this case the fresh leaves were chloroformed before being dried. For the tests a fixed temperature of 77° F. was used. Small samples of the pulverized leaf were placed for 48 hours in desiccators containing saturated solutions of ammonium nitrate, sodium nitrate, ammonium sulphate, and potassium chloride, respectively. The corresponding relative humidities to which the samples were thus exposed were approximately 62.5, 75.1, 81.8, and 85.8 per cent. To ascertain the moisture content of the samples after the exposures they were first weighed, then dried to constant weight over concentrated sulphuric acid, and again weighed. Finally, the samples were heated in the oven at 101° C. for four hours and weighed. Tobacco contains certain volatile constituents other than water, and for this reason the two different methods of estimating the water content were employed. The results are shown in Table 6.

TABLE 6.—*Effect of fertilizers containing a high percentage of chlorine on the water content of tobacco grown at Oxford, N. C., and Upper Marlboro, Md., when exposed at 77° F. to relative humidities corresponding to the vapor pressures of saturated solutions of certain salts*

Locality and treatment or plot No.	Quantity of chlorine in the fertilizer per acre	Chlorine content of tobacco	Water content of tobacco									
			In air-dried condition		Over NH_4NO_3 solution, relative humidity 62.5		Over NaNO_3 solution, relative humidity 75.1		Over $(\text{NH}_4)_2\text{SO}_4$ solution, relative humidity 81.8		Over KCl solution, relative humidity 85.8	
			By drying over H_2SO_4	By drying in oven	By drying over H_2SO_4	By drying in oven	By drying over H_2SO_4	By drying in oven	By drying over H_2SO_4	By drying in oven	By drying over H_2SO_4	By drying in oven
Oxford, N. C.:	Lbs.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Series 1—												
Treatment 1.	0	0.39	3.61	6.02	9.56	14.26	16.55	17.78	19.87	22.37		
Treatment 2.	75	3.58	4.03	7.08	10.78	16.53	19.19	20.78	23.59	26.90		
Series 2—												
1918 (1)-----	0	.52	3.35	5.43	7.89	11.98	13.91	14.70	17.12	19.40		
(2)-----	75	3.15	3.58	5.78	8.44	13.06	15.25	17.25	19.36	21.60		
1925 (1)-----	0	.83	4.43	5.93	8.84	14.35	16.03	18.64	19.97	23.15	24.56	
(2)-----	75	3.19	4.64	6.79	10.35	12.62	17.26	19.50	22.89	24.81	28.62	30.81
Upper Marlboro, Md.:												
Plot 10-----	130	4.80	4.99	6.92	9.75	11.93	14.62	16.72	16.53	18.29	19.81	21.45
Plot 11-----	0	.22	4.91	6.87	9.62	11.32	13.95	15.76	15.77	18.06	18.73	20.71
Plot 18-----	0	.24	5.04	7.80	8.23	11.05	13.85	16.50	16.54	19.26	19.86	22.77
Plot 19-----	0	.23	4.98	7.49	7.56	10.76	12.74	15.84	15.28	17.09	18.27	20.50
Plot 20-----	202	5.34	6.07	9.23	10.92	14.12	17.95	20.42	21.45	22.77	24.56	26.90

In all cases the increased content of chlorine has an appreciable effect on the moisture content of the tobacco, although at relative humidities below 60 per cent this effect is slight. As the relative humidity increases, the effect of the chlorine increases and becomes rather pronounced with a humidity of 75 per cent or higher. Where the green leaf was chloroformed so as to prevent normal curing the effect of the chlorine was much reduced even when the relative humidity was high. Moreover, the data for treatments 1 and 2 of series 2 at Oxford, N. C. (Table 6), suggest that with uniform fertilizer treatment on the same soil there may be a distinct seasonal effect on the hygroscopic properties of the tobacco, the product of 1925 having a greater affinity for water than that of 1918.

The question as to the practical advantage or disadvantage of the increased water content of tobacco resulting from an increased content of chlorine is a matter of some complexity. Much depends on the conditions to which the tobacco is exposed, the use to be made of it in manufacture, and the stage that it has reached in handling and manufacturing operations. As the crop leaves the farm it will ordinarily appear on the market with an increased water content. Under relatively dry conditions the increased water content may tend to give the leaf a better appearance, to increase its elasticity and richness or "oiliness," and to prevent damage from breakage. In damp weather the tendency will be to bring the leaf into very high order and increase the danger of damage from excess moisture. These relationships also will apply more or less to manufactured tobacco products as well as to the leaf. In the case of cigarette, pipe-smoking, and chewing leaf there will be, of course, an increased shrinkage in weight in the redrying process. Finally, it has been found that the moisture content of tobacco materially affects its combustibility, and increased content of moisture is probably one of the factors in the adverse effect of chlorine in the leaf on its combustibility.

The chlorine content often affects the color of the tobacco leaf. As previously noted, a moderate chlorine content tends to produce a lighter shade of green in the leaf in the field, and in the case of flue-cured tobacco the cured leaf often shows a lighter shade of yellow. In the latter case, however, the luster may be somewhat dimmed, the leaf surface showing a more or less faded appearance. These effects are not quite so apparent in most other types of tobacco. With a higher content of chlorine the influence on color of the cured leaf may be decidedly injurious, the leaf surface being more or less splotched with areas of green and yellow and the appearance as a whole distinctly abnormal. The leaf also is often lacking in toughness and elasticity and readily breaks when stretched.

It remains to touch briefly upon the effect of chlorine on the actual market price of leaf tobacco under existing marketing conditions. In the sale of most leaf tobacco, relative values of different grades or qualities of a given type are arrived at by the expert buyer simply by inspecting and feeling the product and on occasion noting its odor. In a broad way, relative values usually, though not always, may be determined with a fair degree of accuracy by this procedure. This is true because the buyer through long experience has learned to associate the characteristics measured by the sense of touch and the color and general appearance of the leaf with certain elements

of quality required in the manufactured product. However, this method of arriving at relative values can not be relied upon to reflect the true worth of the product for manufacturing purposes where the effects of special treatments, such as differences in fertilizer used, are involved, since the buyer is not always in a position to recognize or interpret the effects in question. This applies with special force to differences in combustibility and in aroma and, in some instances, in keeping qualities of the leaf. For example, one of the most valuable characteristics of Maryland tobacco as a whole is its good burning qualities. In tests at Upper Marlboro ammonium chloride has been used at varying rates in the fertilizer in comparison with other forms of nitrogen. In these tests expert buyers have at times assigned the highest valuation to the product grown with ammonium chloride, although the leaf when tested has been found to have such extremely poor burning qualities that it seems probable that if such a fertilizer were used generally in growing the crop the market for this type of leaf would be seriously affected. Moreover, the appearance of the leaf is more or less abnormal, and for this reason the buyers at times have reversed their valuations, assigning a relatively low valuation to this product.

Valuations furnished by experienced buyers are given in Table 1 for flue-cured tobacco grown at Oxford and Reidsville, N. C., over a period of years, with equivalent quantities of sulphate and muriate of potash, with and without the addition of dolomitic or calcitic limestone. The soils in question are deficient in magnesia, and the action of chlorides on the magnesia of the soil already has been discussed. Except where dolomitic limestone was used, the tobacco suffered more or less severely from magnesium deficiency on the plots treated with the sulphate of potash. Injury from this cause was decidedly less marked on the muriate plots in the earlier years of the tests. As an average of 11 comparisons where no limestone was used, the difference in price per 100 pounds of the tobacco was about \$3.12, or 12 per cent, in favor of the muriate. That this difference was due in part to magnesium deficiency rather than to the specific effect of chlorine is indicated by the fact that where dolomite was used seven comparisons gave an average increase in value from the muriate of only \$1.02, or somewhat less than 5 per cent. With use of calcite two comparisons gave but little advantage of the muriate over the sulphate, probably because of the depressing effect of the calcite on the availability of the magnesia in the soil.

As to the quantity of chlorine in the fertilizer required for obtaining leaf of the best quality as judged by appearance, the data in Table 1 are rather limited and afford only a tentative conclusion. Where dolomitic limestone was not used, maximum results as to quality were obtained with quantities of muriate supplying 20 to 30 pounds of chlorine per acre. Where the limestone was applied there seems to have been little or no significant gain in quality from the use of muriate as compared with sulphate. As has been stated, a high chlorine content in the leaf has an injurious effect on its market value as judged by appearance. This effect is illustrated by the valuations made by buyers on the crops of 1926 and 1927 on plots 10 and 11 (run in duplicate) at Upper Marlboro, Md., the treatments of which are indicated in Table 3. The results are summarized in Table 7. In this instance the buyers' valuations for the leaf grown with the chlorides of potas-

sium and magnesium were only about one-half those for the product grown with the sulphates of potassium and magnesium. The reduced valuations were due primarily to the undesirable colors produced by the chlorine.

TABLE 7.—*Effect of a high chlorine content in the fertilizer on the sale value of tobacco grown at Upper Marlboro, Md., as arrived at by buyers after inspection*

Plot No.	Quantity of chlorine in fertilizer per acre	Value of leaf per 100 pounds		
		1926	1927	Average
	Pounds	Dollars	Dollars	Dollars
10.....	130	15.75	14.75	15.25
11.....	0	27.50	31.00	29.25

SUMMARY AND CONCLUSIONS

Although it has long been known that under some conditions common table salt and other chlorides will give measurable increases in crop yields, it has not yet been definitely determined whether chlorine is one of the essential plant nutrients. In the present paper field data are presented which show that on light sandy and sandy loam soils a moderate supply of chlorine in the fertilizer commonly stimulates the growth of the tobacco plant, the average increase in yield obtained experimentally being about 10 per cent. These data are based primarily on tests with sulphate and muriate of potash at rates to supply equal quantities of potash. Where dolomitic limestone was applied to the soil, the gain in yield of tobacco from the chlorine was reduced. Under the conditions of the tests 20 to 30 pounds of chlorine per acre were sufficient to give approximately the maximum stimulating effects of this element. Analyses of the tobacco show that in these tests the potash applied to the soil in the form of sulphate was quite as readily absorbed by the plant as that applied in the form of muriate. However, while the chlorine ion also was readily absorbed by the plant, only a comparatively small proportion of the sulphate ions were taken up. In effect the chloride of potassium may be regarded as entering the plant as such, while the potassium furnished by the sulphate enters largely in some other form. The data indicate that the ease with which the chlorine ion enters the plant is in sharp contrast with the behavior of sulphate and phosphate ions.

It has been found that on the soils employed in these experiments muriate of potash used as a fertilizer results in an increased content of magnesia in the plant. Since these soils are markedly deficient in magnesia, it is probable that the stimulating action of the chlorine in muriate of potash on the growth of tobacco is partly due to its effect in temporarily increasing the availability of the soil's reserve supply of magnesia. It has been observed, in fact, that the immediate effect of the muriate is to reduce decidedly the symptoms of magnesia deficiency in the plant, although in later years this effect may be reversed, as is to be expected where the total supply of magnesia in the soil is low.

The chlorine ion has a marked effect in increasing the water content of the leaf of the tobacco plant through the growing season, thus enabling the leaf to resist injurious desiccation. This is of practical

importance in protecting the leaf against the type of injury known as "drought spot." On light soils of low water-holding capacity this protective action against drought injury may result in adding materially to the commercial value of the crop. The effect of a moderate content of chlorine in the tissues in inducing development of a leaf having a greater spread, a lighter green color, and a smoother surface perhaps may be attributed to the drought-resistant properties imparted by the chlorine. It seems likely that this effect of chlorine in increasing turgidity in the tissues explains, in part at least, the stimulating effect of this element on plant growth which has often been observed in various plants.

While a moderate supply of chlorine in the tissues may be of distinct benefit to the plant in enabling it to resist desiccation and thereby stimulating growth, an excess of this element may seriously interfere with normal carbohydrate metabolism. This effect may be severe enough to check growth, and in the seed bed has been observed to result in the death of young seedlings. Normal amylolytic activity in the leaf is disturbed, with the result that there is marked accumulation of starch and the leaf is greatly thickened. It has been found, moreover, that the water content is abnormally high. The leaf assumes a characteristic appearance, which, as a rule, is easily recognized. This physiological effect of chlorine is possibly a special case of the general principle that the relative concentrations of starch and sugars in the tissues is a function of the water content. Partial loss of water, as in curing, results in rapid depletion of the accumulated starch in the leaf.

The adverse nutritional effects and retarded growth may result from considerably less than 100 pounds of chlorine per acre applied in the fertilizer, and in some cases considerable injury has resulted from only 40 to 60 pounds per acre, although the intensity of the effects from a given quantity of chlorine will necessarily vary with the conditions. Injury from chlorine is most likely to develop or to be intensified on sandy soils having limited buffering properties. On such soils a high content of chlorine in the fertilizer also may cause difficulty in obtaining a good stand of plants. The amount and distribution of rainfall affects both the quantity of chlorine absorbed by the plant and its effect on growth.

Broadly speaking, from the standpoint of quality the chlorine content of the cured leaf may affect its properties either favorably or adversely, depending on the quantity of the chlorine present, the use to be made of the tobacco, and the stage in handling and manufacturing operations which it has reached. The presence of chlorine increases the moisture content of the cured leaf, which may affect more or less its elasticity, its combustibility, and its keeping qualities. Chlorine tends to injure the combustibility of the leaf. This effect is often of great importance but may be more or less obscured by other factors affecting combustibility. A free burn is especially necessary in cigar leaf but is also of considerable moment in the case of cigarette and pipe-smoking types. The adverse effect of chlorine on combustibility is partly due to the fact that its presence reduces the quantity of potash which may exist in the leaf in combination with organic acids. Excess chlorine produces muddy colors in the cured leaf, often with considerable splotching in which green, yellow, and

brown are intermingled. A high chlorine content in the fertilizer tends to injure the toughness and elasticity of the leaf.

As judged by expert buyers on the basis of appearance, leaf tobacco grown with a limited supply of chlorine has shown a moderate increase in market value. Under the conditions of the tests, maximum effects in this direction have been obtained with 20 to 30 pounds of chlorine per acre. It is possible that under some conditions somewhat larger quantities would be required for best results. With an excess of chlorine in the fertilizer, the market value of the leaf has been materially lowered.

To determine the exact quantity of chlorine required in the fertilizer for maximum results as to both yield and quality of tobacco obviously is a complex problem, and a more extensive study of the subject is needed.

LITERATURE CITED

- (1) AMES, J. W., and BOLTZ, G. E.
1915. TOBACCO. INFLUENCE OF FERTILIZERS ON COMPOSITION AND QUALITY. Ohio Agr. Expt. Sta. Bul. 285, p. 173-209, illus.
- (2) ANDERSON, P. J., and SWANBACK, T. R.
1929. REPORT OF THE TOBACCO SUBSTATION 1928. Conn. Agr. Expt. Sta. Bul. 299, p. [145]-203, illus.
- (3) ——— MORGAN, M. F., and NELSON, N. T.
1927. THE PHOSPHORUS REQUIREMENTS OF OLD TOBACCO SOILS. Conn. Agr. Expt. Sta., Tobacco Substa. Bul. 7, 24T p., illus.
- (4) ——— NELSON, N. T., and SWANBACK, T. R.
1928. INFLUENCE OF SOME FERTILIZER INGREDIENTS ON THE BURN OF TOBACCO. Conn. Agr. Expt. Sta., Tobacco Sta. Windsor Rpt. 1927: 18T-35T. (Tobacco Sta. Bul. 10.)
- (5) BARTH, M.
1891. UNTERSUCHUNGEN VON IM ELSASS GEZOGENEN TABAKEN UND EINIGE BEZIEHUNGEN ZWISCHEN DER QUALITÄT DES TABAKS UND SEINER ZUSAMMENSETZUNG. Landw. Vers. Sta. 39: [81]-104.
- (6) BEMMELEN, J. M. VAN
1890. ÜBER DIE ZUSAMMENSETZUNG DER ASCHEN DER TABAKSBLÄTTER IN BEZIEHUNG ZU IHRER GUTEN ODER SCHLECHTEN QUALITÄT INSBESONDERE ZU IHRER BRENNBARKEIT. Landw. Vers. Sta. 37: [409]-436.
- (7) BRUNS, A.
1925. UNTERSUCHUNGEN ZUR AUFFINDUNG DER URSACHE DER AMYLUM VERMINDERUNG-BESCHLEUNIGUNG IN WELKENDENLAUBBLATT. Bot. Arch. 11: 40-103.
- (8) CARPENTER, F. B., and ALLEN, A. H.
1926. EFFECT OF CHLORINE IN TOBACCO. Amer. Fert. 65(7): [21]-24.
- (9) CLAYTON, J.
1688. A LETTER FROM MR. JOHN CLAYTON RECTOR OF CROFTON AT WAKEFIELD IN YORKSHIRE, TO THE ROYAL SOCIETY, MAY 12, 1688. GIVING AN ACCOUNT OF SEVERAL OBSERVABLES IN VIRGINIA, AND IN HIS VOYAGE THITHER, MORE PARTICULARLY CONCERNING THE AIR. In Force, Peter, ed., Tracts and Other Papers . . . 3(12), 45 p. Washington, D. C. 1844.
- (10) COHEN, N. H.
1914. TABAKSGRONDEN EN DE DAAROP GEGROEIDE TABAK. Proefsta. Vorstenland. Tabak [Dutch East Indies], Meded. 11, 11 p., illus.
- (11) CSERHÁTI, A.
1895. VERSUCHE ÜBER DIE BRENNBARKEIT DES TABAKS. Jour. Landw. 43: [379]-458.
- (12) FESCA, M., and IMAI, H.
1888. ÜBER KULTUR, BEHANDLUNG UND ZUSAMMENSETZUNG JAPANISCHER TABAKS. Landw. Jahrb. 17: 329-372.

- (13) FREAR, W., OLSON, O., KRAYBILL, H. R., and ERB, E. S.
1916. TOBACCO EXPERIMENTS. IV. IMPROVEMENT OF BURNING QUALITY OF CIGAR TOBACCO. Penn. Agr. Expt. Sta. Ann. Rpt. 1914/15 (Pt. 2): 331-365, illus.
- (14) FROMME, F. W., and WINGARD, S. A.
1922. BLACKFIRE OR ANGULAR-LEAFSPOT OF TOBACCO. Va. Agr. Expt. Sta. Tech. Bul. 25, 43 p., illus.
- (15) GARNER, W. W.
1907. THE RELATION OF THE COMPOSITION OF THE LEAF TO THE BURNING QUALITIES OF TOBACCO. U. S. Dept. Agr., Bur. Plant Indus. Bul. 105, 27 p.
- (16) ——— BACON, C. W., and FOUBERT, C. L.
1914. RESEARCH STUDIES ON THE CURING OF LEAF TOBACCO. U. S. Dept. Agr. Bul. 79, 40 p.
- (17) HALEY, D. E., NASSET, E. S., and OLSON, O.
1928. A STUDY OF CERTAIN CONSTITUENTS OF THE LEAF AND THEIR RELATION TO THE BURNING QUALITIES OF TOBACCO. Plant Physiol. 3: 185-197.
- (18) HISSINK, D. J.
1905. EINE STUDIE ÜBER DELITABAK. Jour. Landw. 53: [135]-172.
- (19) HOAGLAND, D. R., and DAVIS, A. R.
1925. SUGGESTIONS CONCERNING THE ABSORPTION OF IONS BY PLANTS. New Phytol. 24: 99-111.
- (20) JOHNSON, S. W.
1885. ANALYSES OF TOBACCO LEAF. Conn. Agr. Expt. Sta. Ann. Rpt. 1884: 96-104.
- (21) KOSUTÁNY, T.
1882. CHEMISCH-PHYSIOLOGISCHE UNTERSUCHUNG DER CHARACTERISTISCHEN TABAKSORTEN UNGARNS. 47 p. Budapest.
- (22) LOMANITZ, S.
1924. THE INFLUENCE OF SODIUM CHLORIDE UPON ALFALFA GROWN IN SOLUTION CULTURES. Soil Sci. 18: 353-368, illus.
- (23) LUNDEGÅRDH, H.
1914. EINIGE BEDINGUNGEN DER BILDUNG UND AUFLÖSUNG DER STARKE. EIN BEITRAG ZUR THEORIE DES KOHLENHYDRATSTOFFWECHSELS. Jahrb. Wiss. Bot. 53: [421]-463.
- (24) MAYER, A.
1891. ÜBER DIE VERBRENNLICHKEIT DES TABAKS. Landw. Vers. Sta. 38: [127]-139.
- (25) MOSS, E. G., McMURTREY, J. E., JR., LUNN, W. M., and CARR, J. M.
1927. FERTILIZER TESTS WITH FLUE-CURED TOBACCO. U. S. Dept. Agr. Tech. Bul. 12, 59 p., illus.
- (26) NESSLER, J.
1867. DER TABAK, SEINE BESTANDTHEILE UND SEINE BEHANDLUNG. 150 p. Mannheim.
- (27) ———
1892. ÜBER DEN BAU UND DIE BEHANDLUNG DES TABAKS. Landw. Vers. Sta. 40: [395]-438.
- (28) OGIER, P.
1892. CONTRIBUTION À L'ÉTUDE DE LA COMBUSTION DES TABACS EN FEUILLES. Mem. Manfr. État, Tabacs-Allumettes 2(3): 337-346.
- (29) PATTERSON, H. J.
1894. THE EFFECTS OF DIFFERENT FERTILIZING ELEMENTS ON THE COMPOSITION AND COMBUSTIBILITY OF TOBACCO. Agr. Sci. 8: 329-352, illus.
- (30) PREISSECKER, K.
1906. AUSGEWÄHLTE KAPITEL AUS A. PEZZOLATO'S TECHNOLOGISCHER CHEMIE DES TABAKS. Fachl. Mitt. Österr. Tabakregie 6: 128-138.
- (31) SCHLOESING, [T.]
1860. RECHERCHES SUR LA COMBUSTIBILITÉ DU TABAC. Compt. Rend. Acad. Sci. [Paris] 50: 1027-1030.
- (32) SCHROEDER, H., and HORN, T.
1922. DAS GEGENSEITIGE MENGENVERHÄLTNISS DER KOHLENHYDRATE IN LAUBBLATT IN SEINER ABHÄNGIGKEIT VOM WASSERGEHALT. Biochem. Ztschr. 130: [165]-198.

-
- (33) THORNE, C. E.
1924. THE MAINTENANCE OF SOIL FERTILITY. Ohio Agr. Expt. Sta. Bul.
381, p. 245-354, illus.
- (34) TOTTINGHAM, W. E.
1919. A PRELIMINARY STUDY OF THE INFLUENCE OF CHLORIDES ON THE
GROWTH OF CERTAIN AGRICULTURAL PLANTS. Jour. Amer. Soc.
Agron. 11: 1-32.
- (35) WAGNER, P., DORSCH, R., HAMANN, G., and MÜNZINGER, A.
1908. VERSUCHE ÜBER TABAKDÜNGUNG. Arb. Deut. Landw. Gesell. 138,
99 p.

THE TOXIC CONSTITUENT OF RAYLESS GOLDENROD¹

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INTRODUCTION

Rayless goldenrod or jimmy weed *Aplopappus heterophyllus* (A. Gray) Blake, has for more than 20 years been suspected of causing the sickness known as alkali disease that occurs in western Texas, in the Pecos Valley in New Mexico, and in certain parts of Arizona. This disease was early recognized as clinically identical with the milk sickness of human beings and the trembles of animals, diseases that occur in the Central States and are known to be the result of richweed or white-snakeroot poisoning (5).² Field experiments by Marsh, Roe, and Clawson demonstrated that feeding the New Mexico plant produces a syndrome identical with results from the ingestion of richweed, and established rayless goldenrod as the etiologiical factor in alkali disease (7, 8).

A chemical and pharmacological study of rayless goldenrod was undertaken by the writer, and at the same time richweed was subjected to study. The results of the investigation of the latter plant have been published (2, 3, 4). The poisonous substance responsible for milk sickness and trembles was shown to be an alcoholic compound which was named tremetol. Lack of material prevented a continuation of the study of rayless goldenrod until recently, when a sufficient quantity was obtained to permit the isolation and identification of the active constituent.

METHODS AND MATERIAL

The methods used in this study were the same as those employed in the investigation of richweed previously reported. The plant material was obtained from New Mexico, and except for one lot which was put into milk cans and preserved in denatured alcohol as soon as collected, the plants were air-dried. The extracts from the plants were fractionated and the various fractions were fed to guinea pigs and sheep. Guinea pigs were abandoned as test animals for reasons already given (2) and reliance was placed only on the results obtained from experiments with sheep.

The doses given to sheep were calculated to represent as nearly as possible 16 pounds of dried plant per hundredweight of sheep, given in four doses, one each day, but this standard was departed from at times. If the extract was soluble it was given in water solution; otherwise it was emulsified in water with the aid of alkaline sirup of acacia. All doses were administered by drenching the sheep.

GENERAL PROCEDURE IN FRACTIONATING THE PLANT EXTRACT

In the course of this investigation 11 different lots of *Aplopappus heterophyllus* were extracted and the extracts fractionated by chemical

¹ Received for publication Oct. 2, 1929; issued April, 1930.

² Reference is made by number (italic) to "Literature cited," p. 658.

means in an effort to determine the active principle. In some cases the methods were altered to provide certain special information, but in general the procedure described below was used. Figure 1 indicates the general methods employed.

The ground and dried plant was moistened with alcohol, packed in a tin-lined copper percolator, flooded with alcohol, allowed to macerate for two days, and then extracted by intermittent percolation with alcohol as long as it yielded soluble matter to that solvent. The yield of extract averaged 10 per cent of the material used. The percolate was collected and distilled, the distillate being returned to

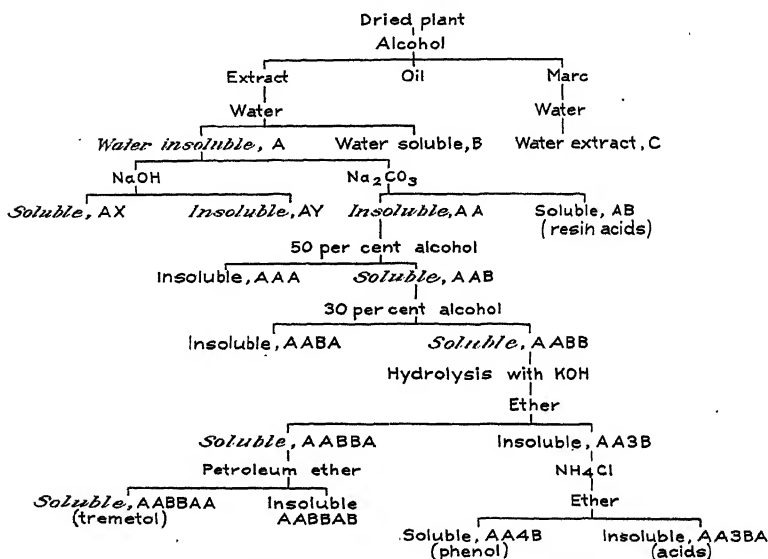


FIGURE 1.—Diagram illustrating the method of fractionating the plant extracts. The active fractions are italicized

the percolator and the residue being frequently poured from the still into a glass receptacle and stored till the end of the process. When the plant had been thoroughly extracted with alcohol the marc was, for one experiment, extracted by water and the water solution was concentrated and used in animal experimentation.

The alcoholic extract was now freed from water-soluble matters by boiling with water in the still, repeating the operation as long as water-soluble substances were removed. This treatment also resulted in the driving off of volatile oil which was condensed and collected. The best yield of oil was from a specimen of plant about a year old and amounted to 0.16 per cent of the weight of the dried plant. The water solutions were collected and concentrated and these constituted fraction B.

The fat and resin portion, insoluble in water, constituted fraction A. In some cases it was further extracted by aqueous sodium carbonate solution which removed resin acids (fraction AB), and in one case aqueous sodium hydroxide was used.

The latter, however, was discarded because it emulsified the tremetol and both resulting fractions were toxic. The fat and resin portion (fraction AA) freed from resin acids was then thoroughly ex-

tracted with boiling 50 per cent alcohol in successive portions, dividing the material into two new fractions, an insoluble portion consisting of fats, waxes, resins, and coloring matters (AAA) and a soluble toxic fraction (AAB).

The dissolved matters were recovered from the latter by distilling off the alcohol when they separated from the watery solution in the form of a thick, brownish, balsamic mass (AAB). This was further fractionated by extraction with boiling 30 per cent alcohol which yielded two fractions, an insoluble and nontoxic portion (AABA) and a soluble toxic fraction (AABB).

The latter was hydrolyzed by boiling with 5 per cent alcoholic potash, the solvent was evaporated off, and the residue was dissolved in water and extracted with several portions of ether. When all had been extracted the ether was distilled off, leaving a brownish oil (fraction AABBA). The KOH solution was then treated with an excess of ammonium chloride and again extracted with ether, which removed phenolic substances (AA4B). The resulting alkaline solution was acidified to recover the acids; the precipitate was filtered off (AA3BA) and the filtrate was extracted by ether to recover soluble acids. The residue from this last ether extraction was added to the acid fraction.

Fraction AABBA contained the tremetol. This was purified by solution in a small quantity of ether and precipitation by four volumes of petroleum ether which threw out much of the coloring matter and contaminating resinous impurities. The ethereal solution was washed with 2 per cent sodium hydroxide solution and then with water, and the solvent was removed. The resulting light-colored oil was redissolved in ether, treated with petroleum ether, dried over fused calcium chloride, and the solvent was removed. This treatment was repeated until the product gave a constant molecular weight, when it was judged pure.

EXPERIMENTS TO DETERMINE THE TOXIC CONSTITUENT

FRACTION C, WATER SOLUBLE

After one lot of plants had been thoroughly extracted by alcohol the marc was extracted by water in order to determine whether the active principle might be insoluble in alcohol and soluble in water. The concentrated water extract was fed to sheep 574 (Table 1) in six doses, each equivalent to 5 pounds of dried plant per hundredweight of animal. There was no apparent effect from this feeding. A similar fraction from another lot was fed to sheep 675 in four doses equal to 4 pounds each per hundredweight without effect.

FRACTION B, ALCOHOL AND WATER SOLUBLE

The water-soluble material obtained by treatment of the alcohol extract as above described reduced alkaline copper solutions, formed a persistent foam, gave Mayer's test for alkaloids, and contained a glucosidal substance that might be precipitated from the solution by making it strongly acid. Four sheep were fed on the whole fraction from different lots. In 1920 sheep 600 and 608 were each given two 5-pound doses without effect. In 1922 sheep 690 was given eight 4-pound doses with the result that it developed some difficulty in breathing and a catarrhal condition that appeared to be due to a traumatic pneumonia. From this the animal recovered. Nothing like trembles was observed.

TABLE 1.—Summary of experimental results of feeding various fractions of the extract of rayless goldenrod to sheep

Dates fed	Animal		Fraction fed	Num-ber of doses	Plant equivalent (lbs. per cwt.)		Character or constituents of fraction fed	Tremetol	Symptoms of animal	Termi-nation
	No.	Weight			Per dose	Total				
1920										
July 1-2	600	Pounds 81	B	2	5	10	Water soluble	None	None	Survival.
July 7-8	608	79	B	2	5	10	do	do	do	Do.
July 10-14	608	72	A B	4	6.15	24.6	Resin acids	do	do	Do.
Aug. 13-24	574	89	C	6	5	30	Alcohol insoluble	do	do	Do.
1922										
July 23-31	690	85.25	B	8	4	32	Water soluble	do	Sick (pneumonia)	Recovery.
July 27-Aug. 1	698	110	A Y	5	4	20	do	Present	Trembles	Death.
Aug. 9-12	676	95.5	A X	4	4	16	do	do	do	Do.
Aug. 6-8	675	108.5	C	4	4	16	Alcohol insoluble	None	None	Survival.
1923										
Oct. 24-27	782	79	A B	4	4	16	Resin acids	do	do	Do.
Nov. 6-8	782	79	A B	4	4	16	do	do	do	Do.
Nov. 14	782	79	A A B	1	8	8	Phenols	do	do	Do.
Nov. 16-20	782	79	A A B	5	4	20	do	do	do	Do.
Dec. 11-14	782	79	A B	4	8	32	Resin acids	do	do	Do.
1924										
May 12-16	782	95	A B	4	4	16	do	do	do	Do.
May 17	782	95	A B	1	8	8	do	do	do	Do.
Sept. 15-18	782	99.5	A A B	4	4	16	Phenols	do	do	Do.
Sept. 22-25	782	99.5		4	4	16	Benzene soluble	Present	Sick	Recovery.
Sept. 26-27	782	99.5		2	8	16	do	do	do	Do.
Oct. 1-6	782	99.5		3	4	12	Benzene insoluble	None	None	Survival.
Oct. 8-23	782	99.5	A A B	17	4	68	Phenols	do	do	Do.
1925										
May 29-June 3	4	60	A A A	4	4	16	50 per cent alcohol insoluble	do	do	Do.
June 8-11	4	60	A A B	4	6.67	26.67	do	Present	Trembles	Recovery.
July 6-11	5	75	A A B A	0	8	0	Acids	None	None	Survival.
July 15-18	5	75	A A B	4	4	16	Phenols	do	do	Do.
July 23-25	5	75	A B	4	32	25	Resin acids	do	do	Do.
Aug. 6-8	5	75		2	6.25	12.5	Vacuum fraction 1	do	do	Do.
Aug. 12-16	5	93		4	11.67	46.67	Vacuum fraction 2	do	do	Do.
Oct. 12-15	5	93		4	16.5	66	Vacuum residue	do	do	Do.
Nov. 3-7	5	93	A A B A A	5	4.3	21.5	Ligroin soluble	Present *	Sick	Recovery.
Nov. 17-20	5	93	A A B A A	4	4.3	17.2	Ligroin insoluble	Present	None	Survival.
Dec. 8-19	5	93	A A B A B	11	7.5	82.5		None	None	Survival.

1926 June 28-July 1-----	6	78.5	AABA	4	18.47	73.88	30 per cent alcohol insoluble.....	do	do	Do.
1927 Mar. 18-22-----	8	57	B	4	4	16	Water soluble.....	do	do	Do.
Do.....	7	94	AB	4	4	16	Resin acids.....	do	do	Do.
July 8-16-----	9	66	AABA	7	5.5	38.5	30 per cent alcohol insoluble.....	do	do	Do.
July 25-29-----	8	72	AABBA	5	4	20	Lignin soluble.....	do	do	Do.
Aug. 1-3-----	8	72	AABBA	3	8	24	do.....	do	do	Do.
Aug. 4-----	8	72	AABBA	1	16	16	do.....	do	do	Do.
July 27-Aug. 5-----	10	60	AABBA	4	5	20	30 per cent alcohol insoluble.....	do	do	Do.

* 1 dose of 3 pounds' equivalent.

The same fraction was fed to sheep 8 in 1927, four doses of 4 pounds being administered without effect. Since these feedings demonstrated that the water solution with its contained glucoside could not be regarded as the causative factor in rayless-goldenrod poisoning, nothing further was done with it.

FRACTION AA, FATS AND RESINS

The failure of the water extracts to produce trembles indicated that the active principle is contained in the fraction insoluble in water. This fraction was divided into two portions by the use of aqueous sodium carbonate solution which dissolved the acid constituents of the mass (fraction AB). The matter insoluble in sodium carbonate was a dark-green, unctuous mass of pleasant, balsamic odor. In one experiment sodium hydroxide was used instead of sodium carbonate to effect the separation, and the two fractions (AX, the soluble matters, and AY, the insoluble) were tested. Fraction AX was given to sheep 676 in four doses, each equivalent to 4 pounds per hundredweight of animal. The animal developed a typical case of trembles, excreting acetone by lungs and kidneys, and died five days after the last dose was administered. Fraction AY was given to sheep 698 in five doses, each equivalent to 4 pounds per hundredweight. On the fifth day the animal began to exhibit symptoms of disease and soon presented a typical case of trembles. Acetone was present in the exhaled air and the urine. The sheep died on the second day after the last dose. These two cases indicated that the active principle was present in the water-insoluble fraction but yielded no information as to whether the principle was acid in nature. It was later learned that the active substance had been emulsified by the alkaline solution and that it could be removed from the mixture by means of ether. Four doses of the fraction after extraction with ether, each equivalent to 4 pounds of dried plant per hundredweight of animal, were given to sheep 782 and did not affect the animal.

FRACTION AB, THE RESIN ACIDS

The resin acids were recovered by acidifying the sodium carbonate solution and filtering off the curdy precipitate that appeared. They constituted a brownish mass of soft, resinous consistence from which nothing of a crystalline nature could be obtained. Resin-acid fractions from several lots were fed at different times. In 1920 four doses, each equivalent to 6.15 pounds of dried plant per hundredweight of animal, were administered to sheep 608 without affecting it. In 1923 sheep 782 received four doses, each equivalent to 4 pounds of dried plant per hundredweight on two occasions, then four doses of 8 pounds' equivalent, and in 1924 the same sheep received four doses of 4 pounds' and one of 8 pounds' equivalent and was not affected. In 1927 sheep 7 received four doses of 4 pounds' equivalent without effect.

Later procedure omitted the fractionation by means of sodium carbonate, and the resin acids were fed in mixture with other constituents. In no case, however, was any effect noted that could be attributed to toxicity on the part of the resin acids.

FRACTIONATION WITH 50 PER CENT ALCOHOL

The resinous mass insoluble in sodium carbonate solution and, in later work, the water-insoluble portion of the original alcohol extract were further fractionated by boiling with 50 per cent alcohol, which dissolved approximately one-third of the whole. The insoluble matter was collected as fraction AAA and the dissolved extract was recovered by removing the alcohol from the solution (fraction AAB). Both portions were tested.

The insoluble fraction AAA was given to sheep 4 in four doses, each equivalent to 4 pounds of dried plant per hundredweight of animal, and produced no effect. The other portion (AAB) was administered to sheep 4 in three doses of 6.67 pounds' equivalent when the sheep became sick. A fourth dose of the same quantity was then administered, and the sheep developed a typical case of trembles. She was given a dose of 2 ounces of Epsom salts in 1 pint of water and fed plenty of oats. She gradually recovered and one week later was discharged. Excretion of acetone was not observed in this case.

FRACTIONATION WITH 30 PER CENT ALCOHOL (AAB)

The active fraction was then thoroughly extracted by boiling 30 per cent alcohol, filtering off the hot solvent. On cooling, a brownish viscous mass was deposited which constituted fraction AAB. The undissolved matter from the original fraction was labeled fraction AABA. The latter was administered to sheep 9 in seven doses, each equivalent to 5.5 pounds of dried plant per hundredweight of sheep, and did not affect the animal. It was also administered to sheep 10 in four doses of 5 pounds' equivalent without effect. Sheep 6 received four doses of this fraction, each equivalent to 18.47 pounds of dried plant per hundredweight, without effect.

FRACTIONATION OF THE ACTIVE MATERIAL (AABB)

The quantity of AABB obtained was too small to permit extensive pharmacological study, so the attempt was made to divide it into constituents and to test these separately. The fraction was dissolved in 5 per cent alcoholic potash and boiled on a water bath under reflex until it was hydrolyzed. The alcohol was then distilled off and the residue was dissolved in water and shaken out with successive portions of ether. This extract (AABBA) contained the tremetol and was purified in the usual way. The solution of ether-insoluble matters was treated with an excess of ammonium chloride to liberate the phenol present and again shaken out with ether. The residual aqueous solution was then acidified and the liberated acids were collected as already described.

THE PHENOL

The phenolic substance isolated from the active fraction was a light reddish-yellow resin which could not be made to crystallize. It decomposed when an attempt was made to distill it in a vacuum of 3 mm. Four doses of it, each equivalent to 4 pounds of dried plant per hundredweight of animal, were given to sheep 782 without effect. A month later 17 doses of the same quantity were given to this sheep without effect. The sheep also received six doses, total-

ing 28 pounds' equivalent of the phenol, from another lot without effect. Sheep 5 received four doses of 8 pounds' equivalent from a third lot without effect. From these feedings it was apparent that the phenolic constituent of the active fraction is not the poisonous agent in the plant.

THE ACID CONSTITUENTS

The acid constituents were collected, washed free from mineral acid, and dissolved in aqueous sodium carbonate for feeding tests. Sheep 5 received five doses of 4 pounds' and one of 3 pounds' equivalent per hundredweight of animal without effect. The fatty acid constituents of the plant also had been administered to sheep in such extracts as fraction AAA and AABA which were not toxic.

THE PETROLEUM-ETHER PRECIPITATE

In the purification of tremetol the ethereal solution of the substance was mixed with 4 volumes of petroleum ether which occasioned a precipitate of resinous matter. This fraction was redissolved in ether and reprecipitated by petroleum ether, freed from solvent, and tested. In 1925 sheep 5 received 11 doses, each equivalent to 7.5 pounds of dried plant per hundredweight of animal, without effect. These experiments made it clear that the active substance is not acid or phenolic in nature and that it is soluble in the ether-petroleum ether mixture referred to.

VACUUM DISTILLATION OF THE ACTIVE FRACTION

Two separate lots of the active fraction were submitted to distillation under reduced pressure of from 3 to 16 mm. There was extensive decomposition, and a large amount of resinous residue remained in the distilling flask. The main fractions of distillate were united and redistilled. The fraction that came over at 175°-188° C. under 1 mm. consisted of a white solid that was recrystallized from alcohol and then melted at 136°-137°. Analyses gave C, 70.78 and 70.88 per cent; H, 6.35, 6.34, and 5.96 per cent. The vacuum distillates and the residue were administered to sheep but did not affect them. It was evident from the character of the distillates that extensive decomposition of the active material had taken place. It may be noted here that prominent among the decomposition products were acetic acid and a terpenelike substance.

THE ISOLATION OF TREMETOL

It was necessary to prepare a larger quantity of the active fraction in order to complete the study of the plant. From a recently collected lot there was obtained a light-yellow oil that resembled the tremetol obtained from *Eupatorium urticaefolium*. This was subjected to analysis and the figures for carbon and hydrogen agreed with those obtained in the case of the former plant. The fraction was then purified until the molecular weight, determined by the freezing-point method with acetic acid as solvent, was constant. The fraction was identified as tremetol by the following characters: Percentage of carbon and hydrogen; molecular weight (found 262,258, calculated 262.17); optical activity, levorotatory, $[\alpha]_D^{30} = -33.82^\circ$; and the color reaction with sulphuric acid was positive.

Tremetol is known to cause the typical symptoms of trembles(4). Its presence in rayless goldenrod is sufficient to account for the cases of range poisoning caused by the plant. No other constituent of the plant was found toxic to sheep, and all of them, with the exception of the volatile oil, were given in sufficient doses to determine their lack of toxicity. The volatile oil is still being studied.

LOSS OF TOXICITY IN DRIED PLANT

One of the most apparent differences between richweed and rayless goldenrod is the fact that the latter is poisonous when dried as well as when green, whereas richweed rapidly loses its toxicity after it is gathered and when dried is incapable of causing trembles, although it may still be poisonous because of its content of toxic resin acid.

The field experiments showed that rayless goldenrod did not lose much of its toxicity on drying and storing for short periods (8). However, the chemical study has shown that rayless goldenrod loses tremetol gradually in storage although at a much slower rate than does richweed. Part of a lot of the former plant extracted 30 months after collection showed a diminished toxicity (sheep 4). Another part of the same lot extracted 55 months after collection contained no tremetol. The extract was worked up to the tremetol fraction and this was administered to sheep 8. Nine doses, equivalent to a total of 60 pounds of dried plant per hundredweight of animal, were given to the sheep. Daily samples of blood were drawn from the left jugular vein. These were tested for the presence of acetone and the amount of blood sugar was determined. The animal showed no effect from the feeding. The fraction fed was later tested for tremetol and that substance was found to be absent. It appeared, therefore, that the plant had lost its toxicity during the four and a half years that had elapsed after its collection.

THE OCCURRENCE OF KETOSIS IN TREMBLES

The appearance of symptoms of ketosis in cases of trembles and milk sickness has been noted especially in richweed poisoning (1). Jordan and Harris (6) published the report of Woodyat, who determined the presence of acetone in urine from milk-sickness patients. In the cases of rayless-goldenrod poisoning that have come under the observation of the writer, ketosis has been an almost constant complication. Acetone has been detected in the exhaled air and in the urine and blood of sick animals and at post-mortem examination. Carcasses of animals dead of the disease often carried a pronounced odor of the ketone. As with richweed poisoning, however, this symptom is not always present and, when absent, the prognosis is more favorable.

SUMMARY

Rayless goldenrod or jimmy weed (*Aplopappus heterophyllus*) causes trembles, known locally as alkali disease, in certain sections of western Texas, New Mexico, and Arizona. The disease as it occurs in these places is clinically indistinguishable from trembles that occurs in the Central States, although in the latter locality the causative plant is richweed or white snakeroot (*Eupatorium urticaefolium*).

Tremetol, $C_{16}H_{22}O_8$, is the toxic constituent of rayless goldenrod. This compound was first isolated from richweed, and is the substance in richweed that causes trembles in animals and milk-sickness in man.

Dried rayless goldenrod appears to lose its toxicity slowly. The process is much less rapid than is the case with richweed.

Animals poisoned on rayless goldenrod develop a ketosis, excrete acetone, become hyperglucemic, and in all respects resemble those poisoned on richweed.

LITERATURE CITED

- (1) COUCH, J. F.
1926. ACIDOSIS, TREMBLES AND MILKSICKNESS. *Science* (n. s.) 64: 456-457.
- (2) ———
1927. THE TOXIC CONSTITUENT OF RICHWEED OR WHITE SNAKEROOT (EUPATORIUM URTICAIFOLIUM). *Jour. Agr. Research* 35: 547-576, illus.
- (3) ———
1928. MILK SICKNESS, THE RESULT OF RICHWEED POISONING. *Jour. Amer. Med. Assoc.* 91: 234-236, illus.
- (4) ———
1928. THE TOXICITY OF TREMETOL. *Jour. Amer. Vet. Med. Assoc.* (n. s. 26) 73: 603-607, illus.
- (5) JORDAN, E. O., and HARRIS, N. M.
1908. THE CAUSE OF MILKSICKNESS OR TREMBLES. *Jour. Amer. Med. Assoc.* 50: 1665-1673.
- (6) ——— and HARRIS, N. M.
1909. MILKSICKNESS. *Jour. Infect. Diseases* 6: 401-491, illus.
- (7) MARSH, C. D., and ROE, G. C.
1921. THE "ALKALI DISEASE" OF LIVESTOCK IN THE PECOS VALLEY. U. S. Dept. Agr. Circ. 180, 8 p., illus.
- (8) ——— ROE, G. C., and CLAWSON, A. B.
1926. RAYLESS GOLDENROD (APLOPAPPUS HETEROPHYLLUS) AS A POISONOUS PLANT. U. S. Dept. Agr. Bul. 1391, 24 p., illus.

METHODS USED IN TESTING MATERIALS AS REPELLENTS AGAINST THE JAPANESE BEETLE¹

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INTRODUCTION

Lead-arsenate sprays are recommended by the Japanese beetle laboratory³ for the protection of late apples, late peaches, shade trees, and foliage of other plants from the attack of the Japanese beetle *Popillia japonica*. There are, however, several favored food plants of the beetle on which lead arsenate can not be used to advantage. Early peaches are very liable to attack by the beetle, but it is inadvisable to spray them with anything that leaves a toxic residue, since, because of the nature of the fruit, the residue can not be removed at the time of harvest. This applies also to early apples, although a certain amount of the residue can be removed from apples by subsequent treatment. The blossoms of flowering plants are severely attacked by the beetle, but a spray which protects by residue alone is effective for only a very brief period, and additional applications are necessary because the leaf and blossom areas are constantly expanding. Various small fruits are attacked by the beetles, and it does not appear at present that a spray or dust could be applied advantageously to such plants. A satisfactory repellent would do much to reduce this type of injury.

The methods described in this paper have been employed in testing over 430 different materials and combinations. As a result of these tests, some materials have been found to possess a definite repellence to the Japanese beetle, and others have been found to be repellent to the beetle only when a certain concentration of their vapor is constantly maintained. Although the problem of finding a satisfactory repellent for the Japanese beetle has not been solved, the methods employed in these tests may be of value to workers engaged on similar problems.

The problem of discovering a material repellent to the Japanese beetle was first undertaken in 1922 at the Japanese beetle laboratory by Loren B. Smith, assisted by T. H. Frison. Since then Mr. Smith has directed the project. The investigations were conducted during 1923 by F. J. Brinley, and during 1924, 1925, and 1926 by E. Avery Richmond. The investigations during 1927 and 1928 were conducted by the writer under the supervision of E. R. Van Leeuwen. As new methods were used in 1927 and 1928, this paper includes only the work done in these years.

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² The writer expresses his appreciation of assistance by L. B. Smith, principal entomologist in charge of the Japanese beetle laboratory, E. R. Van Leeuwen, associate entomologist, E. Avery Richmond, D. H. Grant, and P. A. Van der Meulen, agents, Bureau of Entomology, and I. L. Hunt, jr., temporary assistant.

³ SMITH, L. B. JAPANESE BEETLE CONTROL. N. J. Dept. Agr., Bur. Statist. and Insp. Circ. 90, 31 p., illus. 1925.

LOCATION

The cage tests were conducted in an insectary, and a small number of vaporizer tests were conducted in an apple orchard. The greater part of the work was done at Moorestown, N. J., in a neglected peach orchard, which contains 906 trees, 223 of an early variety and 683 of a later variety. The orchard itself is bounded on three sides by uncultivated fields and on the fourth by an abandoned apple orchard. During the summers of 1927 and 1928 heavy crops of early peaches were almost entirely consumed by beetles as soon as they approached ripeness. In 1927 a heavy crop of late peaches, none of which were injured by the beetle, was ready for harvesting about September 12. There was no crop of late fruit the following year. The entire orchard was heavily infested with beetles during the summers of both years, although the infestation was heavier on the early fruit. Field experiments were begun on July 5, 1927, and concluded August 31. In 1928 field work was begun July 9 and ended August 23, because of diminished infestation toward the close of the season.

METHODS

1. Testing material in comparison with a known attractant.
 - A. In traps.
 - B. In 6-ounce tins.
 - a. Half-hourly observations.
 - b. Continuous observation.
2. Spraying or dusting entire trees.
3. Cage tests.
4. Vaporizer tests.
5. Tests with vapor-dispensing devices.

In testing a large number of substances, of which only a few are likely to prove of value, it is advisable to employ a method that will give reliable preliminary results with a small quantity of material. Since volatile and nonvolatile materials could not be tested by the same method, it was necessary to have one for each group. Method 1 was used in testing materials that were volatile, and method 3 was employed for substances which were practically nonvolatile.

After a material has given favorable results in preliminary tests, it should be tested on a larger scale. Such tests were conducted with volatile materials by methods 4 and 5, while nonvolatile materials were tested by method 2. Method 2 was also used in preliminary tests of nonvolatile substances which could be obtained in sufficient quantity for a thorough application to an entire peach tree.

One ounce of a given chemical is sufficient for a complete test by method 1 or 3. Method 4 requires half a pound, method 2 at least a pound, and method 5 several pounds.

METHOD 1.—TESTING MATERIAL IN COMPARISON WITH A KNOWN ATTRAHENT

This method is based upon the use of the attractants geraniol and eugenol⁴ in comparison with the material being tested to ascertain whether or not the test material decreases the attraction of the geraniol-eugenol combination. In all tests geraniol⁵ and eugenol were combined with a carrier composed of the following ingredients:

⁴ RICHMOND, E. A. OLFACTORY RESPONSE OF THE JAPANESE BEETLE. (POPILLIA JAPONICA NEWM.) Ent. Soc. Wash., Proc. 29: 36-44. 1927.

⁵ The geraniol used in all tests was purchased in one lot, the actual purity of which was found by analysis to be approximately 85 per cent.

Bran, 50 gms.; molasses, 26 c. c.; glycerine, 4 c. c.; water, 9 c. c.; and sodium benzoate, 0.1 gm. This carrier was selected by Richmond from among several combinations because it was the most satisfactory for volatile materials in field tests.

Method 1 appears to give a reliable preliminary indication of the value of a material as a repellent, but such an indication is not always borne out when the material is tested on a large scale. Method 1 also makes possible a large number of tests in a comparatively short time and with very small quantities of material.

MATERIAL TESTED IN TRAPS

Standard traps⁶ were used principally to substantiate conclusions based on results obtained by the use of 6-ounce tins. These traps

had never been used in this connection before, and it was desired to ascertain how useful they would be and if it would be feasible to use them in place of tins. Each material was tested in triplicate. In each series there were three traps containing geraniol together with the material being tested, and three check traps containing geraniol alone. Twenty-five gms. of the carrier, combined with 2.5 gms. of geraniol and 0.25 gm. of eugenol, was placed in one-half of the bait container of each trap. Twenty-five gms. of the carrier, combined with 5 gms. of the material being tested, was placed in the other half of each bait container in three of the traps.

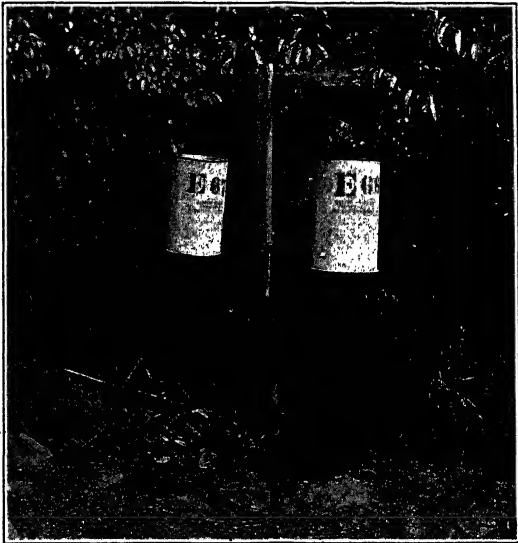


FIGURE 1.—Standard traps used for testing repellents in the field

Posts were set in the ground parallel to rows of peach trees at a distance of 3 feet from the outer branches. All posts were similarly placed with respect to the trees. A trap containing geraniol was hung from one end of a 3-foot cross arm on the top of the post, and one which contained geraniol together with the material being tested was hung from the opposite end. The six traps in each series were located on adjacent posts. Trapped beetles were collected daily. Figure 1 shows the traps as they were set up in the field.

MATERIAL TESTED IN 6-OUNCE TINS

HALF-HOURLY OBSERVATIONS

The 6-ounce salve tins used in these tests were similar to the tins that had been used for several years in the tests of attractants⁷

⁶ RICHMOND, E. A., and METZGER, F. W. A TRAP FOR THE JAPANESE BEETLE. *Jour. Econ. Ent.* 22: 299-314, illus. 1929.

⁷ See footnote 4.

at the Japanese beetle laboratory. Two tins were lashed firmly together side by side to form a double bait can. One tin was filled with 90 gms. of the bran carrier in which 2.5 gms. of geraniol and 0.25 gm. of eugenol were mixed. The other tin was filled with the same weight of the bran carrier to which 5 gms. of the material being tested was added. Each double bait can therefore contained an attractive element, the geraniol-eugenol bait, which was compared with the element being tested. A second double bait can which contained the attractive bait in one tin and the bran carrier alone in the other was used as a check.

Tests were conducted in series. Each series contained three double bait cans treated with different materials, and one check, all of which were hung on one peach tree at equal distances around the periphery and at a height of 4 feet from the ground. All materials were tested in duplicate and the duplicate cans were hung on different trees. Each test was continued until a minimum of 25 beetles had been observed on the check.

The position of each can was changed half hourly according to a prearranged plan. Each of the four positions on a tree was designated by a number, and a tag bearing the number of each position was placed at the proper point. The can which was placed at position 1 was moved to position 2 at the time of the following observation and so on. Nine records were taken daily at half-hourly intervals, from 11 a. m. to 3.30 p. m. At each observation the number of beetles actually present on the cans was recorded.

Results were computed by comparing the number of beetles observed on the treated cans with the number on the respective checks. It was found that when four check cans were placed on the same tree and moved as described above, each attracted approximately the same number of beetles. As the maximum variation was only 12.6 per cent, the results were considered to be sufficiently accurate to serve as a basis for comparison.

CONTINUOUS OBSERVATION

Continuous observations, instead of half-hourly observations, were made during the summer of 1928, and gave better and quicker results.⁸ Otherwise the method was the same, except that a check was prepared for each treated can, and that the cans were hung on stands. One treated can and one check were hung from opposite ends of the cross arm of a stand. (Fig. 2.) They were approximately 3 feet apart and 3 feet from the ground. This distance between cans was selected because it was sufficient to prevent any of the odor of the material being tested from reaching the check can, and the height was determined by considerations of convenience. The stand was placed so that each double bait can was approximately 1 foot from the outer foliage of an infested tree. The preference of the beetle for sunlight was met by placing the cans in the sun. That the odor from each pair of cans should be carried directly into the infested tree was assured by placing them on the windward side. The distance of 1 foot from the outer foliage was considered short enough to allow the full odor to reach the infested foliage, yet long enough

⁸ This method was considered by Richmond in 1926. (Unpublished notes.)

to compel the beetles to fly in order to reach the cans, thus indicating a definite response.

The beetles alighting on each double bait can were counted during a 10-minute period. The position of the cans on the stand was then reversed to equalize, as far as possible, any variation due to the respective positions of the cans. All beetles were then removed and a second 10-minute count made. The 10-minute period was believed to be long enough to allow the influence of the odor to be felt, yet short enough to permit a reversal of position and a second count before any marked change either in the weather or the intensity of infestation on the tree could occur. This was continued until approximately 50 beetles were recorded on the check cans. Less than 50 beetles was considered insufficient for an accurate indication, and more than 50 unnecessary.

Each material was tested three times, 24 hours being allowed to elapse between tests, so that a series of results under varying conditions might be obtained.

RESULTS OF COMPARISONS WITH KNOWN ATTRAHENTS

It should be clearly understood that this method does not give results in terms of direct repellence. It merely shows to what extent a chemical will increase or decrease the attraction of geraniol and eugenol when the material being tested is placed in close proximity to the attractants.

The effect of the material being tested on the attractiveness of the geraniol-eugenol combination may be represented as a quantity, M , derived by the formula:

$$M = \frac{\text{Total number of beetles on treated can or trap} \times 100}{\text{Total number of beetles on check can or trap}}$$

If M equals 0, it indicates that the effect of the material being tested completely counteracted the attraction of geraniol. If M equals more than 100, it indicates that the material being tested increased the attraction of geraniol.



FIGURE 2.—Double bait cans suspended from standard

Three hundred and six different materials were tested by this method, which involved 1,086 tests. The following 45 materials had values for *M* of less than 25 in all cases and include all those substances which might be expected to repel beetles when used alone. The materials are arranged according to the degree by which they decreased the attraction of the geraniol-eugenol combination, *o*-cresol being superior to all others in this respect:

O-cresol, pine-tar oil, phenol, Dippel's oil, high-boiling tar acids (Welsh), coal-tar neutral hydrocarbon oil, trichlorobenzene, crude dichlorobenzene No. 1, alpha chloronaphthalene, crude dichlorobenzene No. 2, white creosote from coal tar, destructively distilled pine wood oil, methyl anthranilate, xyldine, proprietary disinfectant No. 1, guaiacol, crude coal-tar creosote, methyl salicylate, carvacrol, cyclohexyl acetate, *p*-bromophenol, dimethyl aniline, *p*-chlorophenol, discard oil (from Dippel's oil), naphthalene, quinoline, ethyl chloroacetate, *o*-bromophenol, quinaldine, hyssop oil, alpha naphthol, creosote carbonate, ethyl benzoate, furfuryl alcohol, vanillin, *o*-chlorophenol, furfural and pine-tar oil mixture, acetaldehyde ammonia, cresylic acid (Welsh), cade oil, Carolina heavy pine tar oil, safrol, cajeput oil, acetone, and cascarilla oil.

A rough classification of these materials is given in Table 1. This table also gives the total number of substances in each group that were tested, and the number and percentage of those having a value under 25 for *M*, together with the number and percentage of those of each group having values over 100. The limitations of such an arrangement are obvious, since the composition of many of the substances is complex and incompletely known. The classification is included only to illustrate certain facts regarding the value of method 1.

TABLE 1.—*Classification of materials having values of 25 or under and of 100 or over for M*

Chemical group ¹	Materials tested	Materials having <i>M</i> value under 25		Materials having <i>M</i> value over 100	
	Number	Number	Per cent	Number	Per cent
Acids, organic.....	5	0	0	3	60.0
Alcohols.....	22	1	4.5	5	22.7
Aldehydes.....	19	2	10.5	3	15.8
Amino compounds.....	11	4	36.4	2	18.2
Balsams, oleoresins.....	5	0	0	1	20.0
Coal-tar creosotes.....	7	7	100.0	0	0
Cyclic nitrogen compounds.....	5	2	40.0	1	20.0
Empyreumatic oils.....	11	7	63.6	0	0
Essential oils.....	88	3	3.4	15	17.0
Esters.....	41	6	14.6	12	29.2
Ethers.....	20	3	15.0	3	15.0
Fatty oils.....	3	0	0	2	66.6
Halogenated organic compounds.....	40	10	25.0	1	2.5
Hydrocarbons.....	20	2	10.0	1	5.0
Ketones.....	17	2	11.8	6	35.3
Nitro compounds.....	1	0	0	0	0
Phenols.....	20	12	60.0	1	5.0
Plant extracts and juices.....	7	0	0	4	57.1
Sulphur compounds.....	8	0	0	1	12.5

¹ A number of chemicals possess the functions of 2 or more groups and are included in each, thus causing duplication.

It will be seen in the table that the coal-tar creosotes and empyreumatic oils, as groups, had the lowest *M* values. In all the coal-tar creosotes tested, the value of *M* fell below 25, and 7 of the 11 empyreumatic oils had values under that figure. The phenols and cyclic nitrogen compounds had the next higher values in this group. On the other hand, only a small percentage of the materials in certain other groups, such as essential oils, esters, and ketones, had values under 25, while a much greater percentage had *M* values of over 100.

The purpose of the preliminary tests was to obtain an indication of the value of a large number of materials in as short a time as possible. The method under discussion met this qualification and has proved especially valuable in the search for a repellent for the Japanese beetle.

The trap method and the continuous observation method are far superior to half-hourly observations and are equally effective.

In a number of cases, certain substances have greatly reduced the attractive power of geraniol when tested by method 1, but when used in spray, dust, or vapor form, on or near flowering plants, have afforded little or no protection from beetle attacks. As the concentration of vapor was known to be less when the material was used on a large scale than when it was used in comparison with geraniol and eugenol in 6-ounce tins or traps, it appears that satisfactory results with volatile materials can be obtained only by maintaining a concentration of repellent vapor which is comparable to that which was obtained in the trap or double bait-can tests.

METHOD 2.—SPRAYING OR DUSTING ENTIRE TREES¹

Two peach trees of approximately the same size, and located in the same part of the orchard, were used for each test in 1927. There was sufficient space between them to prevent a material applied on one from affecting the beetle infestation on the other, and trees were chosen on which the beetle infestation was approximately the same. One of the trees was treated with the material being tested, and the other was sprayed with water only to serve as a check. The beetles on each tree were counted before and after spraying. Treatments were made as soon as the beetles became active in the morning, usually between 9 and 10 o'clock. Counts of the number of beetles present on each tree were made at 15-minute intervals throughout the remainder of the day until 3 p. m. If a material still appeared to be repellent at the end of the first day, a single observation was made each day thereafter until no repellence was evident.

Ratios for the treated and check trees were obtained by dividing the average infestation computed from the 15-minute counts by the original number of beetles present on the tree before the test was begun. The repellence for each material was considered to be the difference between the ratio for the treated tree and the check.

In 1928 a large number of the sprays and dusts were applied to early peach trees, which bore a fair crop of fruit. Treatments were made before any beetle injury was noted on either fruit or foliage. Several materials were applied after the trees were infested, and a number of tests were begun after the fruit was gone. Check trees were left untreated when no beetle injury was noted, but in the tests started after the insects became numerous the check trees were sprayed with water alone. Ten tests were usually begun at one time, and two checks, together with a tree dusted with 1 to 9 lead arsenate and hydrated lime, were included in each series.

If a tree was infested with beetles when it was treated, a count was made of the number before and after the treatment was applied. Daily observations were made on all treated and check trees as long as a material showed any repellence, or as long as was necessary in order to obtain an indication of the value of the material.

¹ This method is a modification of that used by Richmond prior to 1927.

Almost all the materials tested during 1928 were nonvolatile or nearly so. The purpose of these tests was to ascertain the repellence of residues of varying conspicuousness. Some materials were hardly visible after application, whereas others left a very conspicuous coating on the foliage.

This method was employed as the final step in the determination of the repellent value of materials in spray or dust form. As the procedure is similar to that followed in practical control work, definite and final conclusions may be made as to the particular value of each material tested. Some of the results are given to illustrate the value of this method.

RESULTS OF SPRAYING OR DUSTING ENTIRE TREES

RESINS, OLEORESINS, AND BALSAMS

Resins, oleoresins, and balsams were emulsified and used at the rate of 1 part of the actual material to 128 parts of water. Wheat flour was added as a spreader at the rate of 2 pounds to 50 gallons. All the materials adhered well to the foliage and left only a slightly conspicuous residue.

Fifteen materials applied before infestation on early peach trees which were bearing fruit delayed the infestation of the fruit from one to four days and gave considerable protection to the foliage as compared with the fruit and foliage injury on the untreated checks. The fruit was not protected for a sufficient length of time for the treatments to be of practical value. Included in this group of materials and listed in the order of decreasing repellence, are the following: Gamboge, labdanum, olibanum, orris root, and scammony resins, Canada fir balsam, elemi resin, tonka oleoresin, styrax (American), ammoniac, benzoin (Siam), and styrax (Asiatic) resins, male fern oleoresin, and myrrh resin. Five other substances of this nature had practically no effect in preventing infestation either of fruit or of foliage.

Guaiac resin, pine-needle extract (German), and Peru balsam were applied to infested trees bearing fruit. These materials decreased the infestation on the foliage of the trees to which they were applied but afforded no protection to the fruit, which was consumed almost as soon as that of the check trees. Cubeb oleoresin, which was applied at the same time, failed to give any protection to fruit or foliage.

Oleoresin of black pepper was the only material of this group that was tested on an infested tree on which there was no fruit. It gave excellent protection to the foliage.

PHARMACEUTICAL PLANT EXTRACTS

Commercial United States Pharmacopoeia or National Formulary extracts of drug plants were used at the rate of 1 part to 64 parts of water, with 0.2 part of a 10 per cent saponin emulsifier. Flour was added as a spreader at the rate of 2 pounds to 50 gallons of water, and fish oil was added as a sticker at the rate of 1 part to 500 parts of water.

Eleven materials were applied on uninfested trees which were bearing fruit, 8 on infested trees with fruit, and 49 on infested trees without fruit. None of these materials retarded or decreased beetle infestation on fruit or foliage, as compared with untreated checks.

The results indicate that certain materials of a resinous nature, which leave little perceptible residue but which stick well to fruit and foliage of peach trees, have much better repellent properties than any of the pharmaceutical extracts which were tested at the concentrations given above.

METHOD 3.—CAGE TESTS

The value of certain materials as repellents for the Japanese beetle was tested under cage conditions during the summer of 1927. Volatile materials were used almost exclusively, and the results were unsatisfactory. Since the cage method has several advantages over field methods it was decided to use it during the summer of 1928, but only in testing nonvolatile materials. Most of the materials tested could not be used in the field because of the small quantity available.

The cages were similar to those used in insecticide studies at the Japanese beetle laboratory for several years. They were 24 inches long, 12 inches wide, and 14 inches high. Bottom and ends were of wood, with a hinged door at one end to permit the introduction of insects and plants. (Fig. 3.)

All materials were sprayed or dusted on newly collected peach twigs, which were kept in a fresh condition by placing them in 2-ounce bottles of water held upright on the floor of the cage by brackets. Fifty beetles were used in each cage. Each experiment lasted four days, unless most of the beetles died sooner, and observations were made every 24 hours.

When a plant in the field has been treated with a material repellent to the beetle, the insect merely ignores it and selects an untreated plant. In order to approximate field conditions, treated and untreated twigs were used in the same cage, and the degree of repellence was determined from the number of beetles feeding on the treated twigs. As a further test for each material, treated food was used alone in other cages to ascertain whether beetles would feed on it when no untreated food was available.

For each material there were 2 cages containing both treated and untreated twigs and 2 cages containing treated twigs only. Tests were made in series, each of which consisted of five groups of 4 cages each, treated as described above, one group of 4 cages containing twigs dusted with lead arsenate and hydrated lime (1 to 9), and 2 cages containing untreated twigs to serve as checks.

All materials tested by this method are practically nonvolatile, and any repellence must have been caused by the presence of a residue. Deposits left on the foliage by the different treatments were of varying conspicuousness.

The result of each test was obtained by comparing the total number of beetles feeding on the treated twigs during the entire course of the

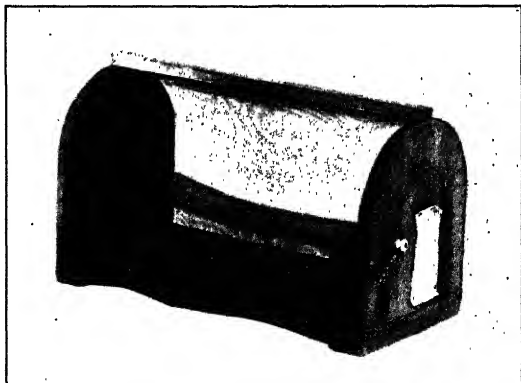


FIGURE 3.—Cage employed to test repellents by method 3

test with the number feeding on the respective checks. The effect of the material in repelling beetles may be represented as a quantity, M or N, derived as follows:

$$M = \frac{\text{Number of beetles feeding on treated twigs} \times 100 \text{ (in cages containing both treated and untreated twigs)}}{\text{Number of beetles feeding on untreated checks}}$$

$$N = \frac{\text{Number of beetles feeding on treated twigs} \times 100 \text{ (in cages containing treated twigs only)}}{\text{Number of beetles feeding on untreated checks}}$$

RESULTS OF CAGE TESTS

When the value of M fell below 25, the material was considered to be repellent to the beetles. When the value of N fell below 25, the

repellence was considered to be still greater. A good example is afforded by the lead arsenate-hydrated lime treatments. In 15 tests the average value for M was 17, and that for N was 19, which indicates clearly that this dust was strongly repellent to the beetle. Numerous applications of this dust in the field have also demonstrated its value as a repellent. Since the results from its use in the cages were similar to those in the field, it appears that data obtained by this method are a good indication of similar results in the field.

When the value of M was less than 25 and the value of N was greater, it was considered as an indication that the material would

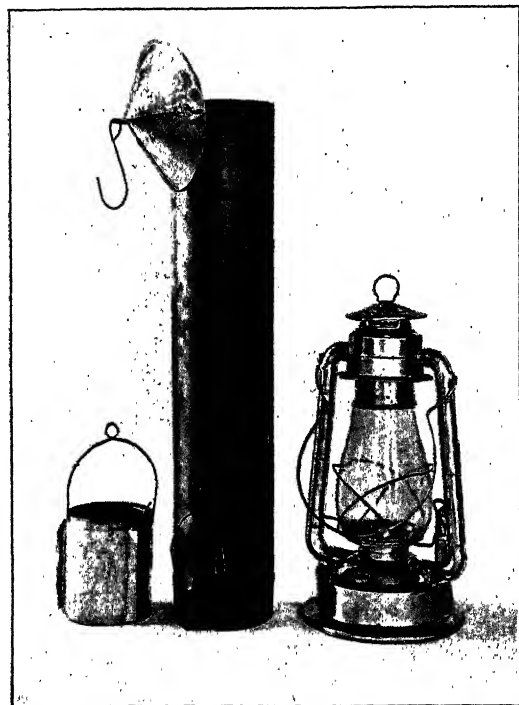


FIGURE 4.—Vaporizer (unassembled) used in testing repellents by method 4

probably be repellent if abundant untreated foliage were available, but that it would not prevent feeding if the beetles were hungry.

If the values of both M and N are greater than 25, the material has little or no repellent value.

METHOD 4.—VAPORIZER TESTS

Vaporizers were used for applying constant heat to certain chemicals to increase the rapidity of volatilization. The vaporizer¹⁰ which

¹⁰ This apparatus was devised by P. A. Van der Meulen and F. E. Mehrhof, agents, Japanese beetle laboratory.

proved most satisfactory under field conditions (fig. 4) consisted of a 30-inch length of 5-inch stovepipe and a lantern with its chimney. The stovepipe was fitted over the top of the lantern so as to cover all but the lower portion of the chimney and was fastened to the lantern with heavy wire. As the flame of the lantern was not exposed to air currents, there was no danger of its being blown out. The reservoir contained sufficient oil for burning 24 hours. The chemical being tested was placed in a can which was held in the end of the stovepipe by three friction fins and could be raised or lowered by means of a heavy wire bail. A cone of galvanized iron was attached 1 inch above the top of the stovepipe to protect the chemical from rain. The vaporizer was suspended from a heavy wire hook above the cone.

The vaporizers were placed in the orchard between 9 and 10 a. m., after the beetles had become active at a place where there was a heavy beetle infestation, so that if the material proved to be repellent, the action would be particularly noticeable. They were taken in at 3 p. m. When used in the orchard the vaporizer was placed in the outer branches to windward of the tree, with the top usually 4 to 5 feet from the ground. In several tests the vaporizers were placed near clumps of evening primrose and rows of marigolds, which grew to a height of 3 to 5 feet. The apparatus was then suspended from a 4-foot standard (fig. 5) on the windward side of the plants, with the top of the vaporizer slightly below the highest shoots.

The apple trees were very large, and the vapor could not reach the entire leaf area. The effect was, therefore, more or less localized. As the peach trees were smaller, the vapor could easily reach all parts of the tree. The infestation was very heavy on both apple and peach trees, and, as an accurate count of the beetles was impossible, the only indication of the repellence of a material was a marked decrease in the infestation.



FIGURE 5.—Vaporizer shown in Figure 4, in operation

RESULTS OF VAPORIZER TESTS

Thirteen materials were tested by this method, and each was used on at least two different occasions. Eight substances, carvacrol, o-chlorophenol, coal-tar neutral hydrocarbon oil, o-cresol, Dippel's oil, methyl anthranilate, pine-tar oil, and quinoline, reduced the infestation by at least 75 per cent in each test. All of these materials

greatly reduced the attraction of geraniol and eugenol in tests by method 1. Only one substance (a crude chloronaphthalene) which had a value for M of less than 25 in method 1 failed to show marked repellence when tested in a vaporizer.

The results obtained by the use of this apparatus indicate that the method is a good one, and, for the first time since the project was organized, it appears possible to maintain effective repellence by means of a volatile material.

METHOD 5.—VAPOR-DISPENSING DEVICES

Liquid-dispensing bottles (fig. 6), designed by P. A. Van der Meulen, were used during the summer of 1927. Each bottle was

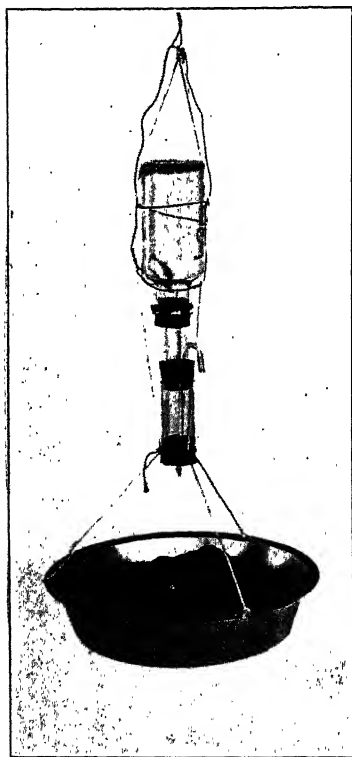


FIGURE 6.—Liquid-dispensing bottle used to test repellents by method 5

suspended from a peach tree, care being taken to have the apparatus located as near the center as possible. A tin pan containing a large sponge was hung directly under the bottle so that the liquid falling from the bottle would be caught on the sponge. A tree in the immediate vicinity of each tree bearing a bottle was used as a check. The trees were of an early variety. The fruit was approaching ripeness, and fruit and foliage were heavily infested with beetles when the tests were begun. None of the materials gave satisfactory results when tested in this apparatus.

During 1928 each material was tested on two peach trees bearing fruit, and all tests were begun before the trees were infested. Solid materials were mixed with bran or sawdust and broadcast on the ground under a tree. They were also placed in cylindrical 16-mesh wire-screen baskets of 1-pound capacity and suspended from the branches. Five baskets were used for each tree, and were distributed about the tree at a height of 5 to 6 feet, well in toward the trunk. Liquid materials were placed in dispensing bottles, or mixed with bran and broadcast under the trees, or absorbed in balls of cheap

cotton batting.¹¹ Five of these balls were suspended from each tree in the same manner as the wire baskets. Two dispensing bottles were used for each tree, and were located several feet apart on opposite sides of the trunk at a height of 5 to 6 feet.

Treatments were repeated at weekly intervals, or more often if the odor could not be noticed around the tree. The average quantity of each chemical was 5 pounds per treated tree. Each chemical was used both on the ground under the tree and in the foliage, and two trees were used in each test.

¹¹ Cotton balls were first employed by Richmond in 1926.

RESULTS OF TESTS WITH VAPOR-DISPENSING DEVICES

Six of the materials tested, o-cresol, Dippel's oil, alpha naphthylamine, phenol, pine-tar oil, and quinoline, afforded excellent protection to peach foliage and retarded the infestation of fruit from one to three days, as compared with the untreated checks. Five of these substances had a value of less than 25 for M when tested by method 1. Alpha naphthylamine had a value of 37. The results of the tests with vapor-dispensing devices are a further check on the accuracy of method 1.

While the vapor of the various materials was sufficiently strong to protect foliage, it did not prevent infestation of the fruit, which took place almost as soon as it did on the check trees. This method provides a means for using volatile materials in other than spray or dust form but can be employed only with comparatively cheap substances.

SUMMARY

The five methods discussed in this paper were used during 1927 and 1928 in testing 430 materials, alone and in combination, as repellents for the Japanese beetle. More than 1,500 tests were conducted.

Tests of volatile materials in comparison with known attractants, as conducted by method 1, give indication of the probable value of such substances as repellents in a comparatively short time, and with the use of a small quantity of each material. This method indicates, further, that certain groups of chemicals are much more likely to be repellent to the beetle than others.

The use of method 2, in which entire trees are sprayed or dusted, presents a means for testing volatile and nonvolatile materials on a scale comparable to that of practical control operations.

Method 3 makes it possible to ascertain the value of a nonvolatile material as a repellent under cage conditions with a minimum expenditure of time and material.

Vaporizers, used in method 4, furnish a useful means for increasing the vapor concentration of volatile materials and of testing the value of such substances as repellents.

A number of miscellaneous vapor-dispensing devices, employed in method 5, failed to give satisfactory results when used with certain materials.

THE PROPERTIES OF ARACHIN AND CONARACHIN AND THE PROPORTIONATE OCCURRENCE OF THESE PROTEINS IN THE PEANUT^{1 2}

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INTRODUCTION

The importance of peanuts both as an article of human food and as a feedstuff is becoming generally recognized. The high nutritive value of the proteins of peanuts has been shown in previous publications from this laboratory, and elsewhere. Peanut press cake has been shown to be an excellent protein concentrate to supplement rations containing proteins deficient in essential amino acids.

Knowledge regarding the proteins of the peanut is limited almost entirely to that contained in an early publication by Ritthausen (6)³ and in a more recent series of articles from the Bureau of Chemistry and Soils of the United States Department of Agriculture. Ritthausen extracted oil-free peanut meal with various saline and alkaline solutions and precipitated the globulin fraction by dilution with water, and by acidification with acetic and sulphuric acids. No evidence was obtained indicating the presence of more than one globulin. In the investigations carried on in the Bureau of Chemistry, Johns and Jones (1), in 1916, isolated from peanut meal two globulins which they named arachin and conarachin. The arachin was precipitated from a 10 per cent sodium chloride extract of the meal (a) by dilution with water, (b) by making the salt extract 20 per cent saturated with ammonium sulphate, and (c) by saturating the salt extract with carbon dioxide. The more soluble conarachin was precipitated by dialyzing the filtrate from the arachin, or by saturating it with ammonium sulphate. Arachin was obtained in much the larger quantity and different from conarachin in having only about one-third as much sulphur, and in containing less basic nitrogen. Besides arachin and conarachin a trace of a substance having the properties of an albumin was isolated. The quantity, however, was so small that its nature could not be definitely established. No evidence was obtained indicating either the presence or the absence of proteins of the prolamin or glutelin type. Later, studies of the chemical composition of arachin and conarachin were made, including a study of the distribution of the nitrogen by the Van Slyke method (2), the determination of certain amino acids by colorimetric methods (4), and the hydrolysis of arachin with subsequent determination of the resulting amino acids by the Emil Fischer ester method (3).

In these studies attention was confined chiefly to the chemical character of the two globulins. There were no quantitative data

¹ Received for publication Dec. 31, 1929; issued April, 1930.

² Constructed from a dissertation presented by Millard J. Horn in June, 1929, to the faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of doctor of philosophy.

³ Reference is made by number (italic) to "Literature cited," p. 682.

regarding the proportion in which arachin and conarachin occur in the peanut. The methods available for the isolation and preparation of proteins in a condition satisfactory for their characterization usually require the sacrifice of yields for the sake of purity. Even though the amino-acid composition of the individual proteins in a given food product is known, it is obviously impossible to estimate the percentages of amino acids in the whole material unless data are available regarding the relative proportions in which the proteins occur. This is an important consideration from a practical standpoint. The dietitian or animal husbandman is chiefly interested in knowing how much of the nutritionally essential amino acids the whole food or feedstuff contains rather than the percentages of amino acids in the individual proteins. The investigation described in this paper was undertaken to obtain more information regarding the physical properties of arachin and conarachin, as well as further evidence showing whether peanuts contain proteins other than globulins, and whether they contain nitrogen of a nonprotein nature.

PREPARATION OF THE MEAL AND EXTRACTION OF THE GLOBULINS

Raw, shelled Virginia peanuts were ground in an electrically driven mill, and most of the oil was removed by two or three extractions with petroleum ether. The meal was again ground as fine as possible, and the residual oil removed by repeated extractions with ethyl ether. The meal thus prepared contained 7.36 per cent of nitrogen, equivalent to 40.48 per cent of protein ($N \times 5.5$, the conversion factor for peanut proteins).⁴

Fifty grams of the meal was shaken for three or four hours in a 500 c. c. centrifuge bottle with 250 c. c. of 10 per cent sodium chloride solution. The suspension was then centrifuged, and the supernatant liquid decanted. The residue was repeatedly extracted in a similar manner with fresh portions of salt solution until no more nitrogen was removed. The extracts were combined, filtered, and diluted to 2 liters with 10 per cent salt solution. This is hereafter referred to in this paper as the extract. For quantitative work on the peanut proteins it is necessary to use freshly prepared extracts. After the saline extracts have stood for about three days, even in an ice box, changes take place, resulting in the precipitation of a light material and the formation of nonprotein nitrogen, which increases in quantity as the extract continues to stand. After a 10 per cent sodium chloride solution of pure arachin had stood in the ice box for several days, it was found that material coagulating at three different temperatures could be separated from the saline solution. The precipitation limits with ammonium sulphate were also changed. With an old solution of arachin a clear-cut separation between arachin and conarachin could not be effected. Small quantities of material separated at 50 per cent, 60 per cent, and 70 per cent of saturation with ammonium sulphate. Nitrogen determinations on aliquots of the extract showed that it contained 86 per cent of the total nitrogen in the meal.

⁴ Inasmuch as arachin and conarachin represent more than four-fifths of the total nitrogen in peanuts, and since both contain 18.29 per cent nitrogen, the figure 5.5 is used as the factor for converting the percentage of nitrogen into that of protein.

PRELIMINARY TESTS ON THE EXTRACT

Coagulation tests were made by heating 10 c. c. portions of the extract in a test tube immersed in a water bath, the temperature increasing at the rate of about 4° C. per minute. The first coagulum appeared at 80° C. This was filtered off, and the clear filtrate on further heating became somewhat cloudy at 94°, but no coagulation occurred even when the filtrate was heated to boiling temperature. When the hot solution was slightly acidified with acetic acid, however, a copious precipitate formed.

Precipitation tests with ammonium sulphate likewise showed no evidence of the presence of more than two proteins. Measured quantities of a saturated solution of ammonium sulphate were successively added to 10 c. c. of the extract so as to raise the concentration of ammonium sulphate in the extract by increments of 10 per cent of saturation. After each addition any precipitate formed was filtered off, and another portion of ammonium sulphate added to the clear filtrate. The first appearance of cloudiness occurred at about 20 per cent of saturation, and a gradual increase in the precipitate formed was obtained up to 40 per cent of saturation. Beyond that there was no precipitation until a fraction separated between 70 and 80 per cent of saturation. Continued addition of ammonium sulphate to the filtrate from this fraction up to complete saturation caused no further precipitation. The final stage in completely saturating the filtrate was accomplished by addition of solid ammonium sulphate.

From the results of the coagulation and precipitation tests it was concluded that the extract contained only two proteins.

PROPERTIES OF ARACHIN

When separated from a salt solution by precipitation with ammonium sulphate, or by dilution with water, arachin settles out as a sticky, heavy precipitate. By stirring this precipitate with gradually increasing concentrations of alcohol, and finally dehydrating it with absolute alcohol and ether in the usual way, arachin can be obtained in the form of a fine, white powder. Arachin can also be precipitated from a 10 per cent sodium chloride solution by adding water until a slight cloudiness appears, and then saturating the liquid with carbon dioxide gas.

COAGULATION TEMPERATURE

For the coagulation tests a fresh solution of arachin was prepared as follows: A fresh, filtered, 10 per cent sodium chloride extract of the peanut meal was made 40 per cent saturated with ammonium sulphate. The precipitated arachin was redissolved in salt solution, and reprecipitated by dilution with 10 volumes of water. From half a gram to a gram of the moist precipitate was dissolved in 20 c. c. of 10 per cent sodium chloride solution.

Ten cubic centimeter portions of the arachin solution when slowly heated began to show cloudiness at 90° C., but no precipitate formed even when the solution was boiled. When the hot solution was slightly acidified with acetic acid a heavy precipitate formed.

SPECIFIC ROTATION

For determining the specific rotation of arachin a 10 per cent sodium chloride solution was used. The average of several closely agreeing polarimetric readings gave $[\alpha]_{\frac{20}{D}} = -39.5^\circ$. (Table 1.)

TABLE 1.—Optical rotation of arachin in 10 per cent sodium chloride solution

Arachin in solution	Angular rotation	Specific rotation ^a
<i>Grams per c. c.</i>	<i>Degrees</i>	<i>Degrees</i>
0.0307	-1.2129	-39.5
.00880	-.34657	-39.3
.04085	-1.5595	-38.1
.0205	-.7971	-38.9
.0192	-.7971	-42.5
		Av. -39.5

^a $[\alpha]_{\frac{20}{D}} = \frac{\alpha}{l \times w}$ where α = specific rotation, l = length of tube, w = weight of protein in grams per cubic centimeter, and α = reading in angular degrees.

PRECIPITATION LIMITS WITH AMMONIUM SULPHATE

Ten cubic centimeters of a 10 per cent sodium chloride solution containing 2 per cent of arachin was treated with trichloroacetic acid. The precipitate was filtered off, and the filtrate examined for nitrogen. No nitrogen could be detected. Measured quantities of a saturated solution of ammonium sulphate were then successively added to 10-c. c. portions of the solution. The precipitates were filtered off, and trichloroacetic acid was added to the filtrates. The smallest quantity of ammonium sulphate which had to be added so that the filtrate would give no turbidity with trichloroacetic acid, thus showing complete precipitation of the arachin, was found to be at 40 per cent of saturation. Most of the arachin was precipitated at 30 to 32 per cent of saturation.

PROPERTIES OF CONARACHIN

Besides the differences in their chemical composition as noted in previous publications (1, 2), the two peanut proteins are distinguished by marked physical differences. Conarachin is much more soluble than arachin. It can not be precipitated from its salt solution by dilution with water without the use of enormously large volumes. It also differs greatly from arachin in its coagulation temperature and precipitation limits with ammonium sulphate and in specific rotation.

COAGULATION TEMPERATURE

Solutions of conarachin were prepared for the coagulation tests as follows: The arachin was first separated from a fresh salt extract of the meal by precipitation with ammonium sulphate (40 per cent of saturation), and the filtrate dialyzed in parchment bags against running water for 20 days, toluene being used as a preservative. The clear dialysate was decanted, and the precipitated conarachin was washed with distilled water and dissolved in 10 per cent sodium chloride solution (1 gm. moist precipitate per 40 c. c. of solvent).

When the conarachin solution was heated slowly the globulin flocculated at 80° C. On being heated to boiling the filtrate remained clear.

SPECIFIC ROTATION

Specific rotation determinations made on 10 per cent sodium chloride solutions of conarachin gave an average of -42.7° . (Table 2.)

TABLE 2.—*Optical rotation of conarachin in 10 per cent sodium chloride solution*

Conarachin in solution	Angular ro- tation	Specific rota- tion
<i>Grams per c. c.</i>	<i>Degrees</i>	<i>Degrees</i>
0.00898	-0.38122	-42.4
.00149	-.19330	-43.0
		Av. -42.7

PRECIPITATION LIMITS WITH AMMONIUM SULPHATE

This determination was similar to that described for arachin. No precipitation occurred on addition of ammonium sulphate until 80 per cent of saturation was reached. All the conarachin was precipitated at 80 to 85 per cent of saturation.

CHARACTER OF THE NITROGEN IN THE PEANUT EXTRACT

Before proceeding with the quantitative determinations of arachin and conarachin it was necessary to study the behavior of the peanut proteins toward certain protein-precipitating agents, in order to ascertain whether all the nitrogen in the extract represented intact protein molecules,⁵ or whether the extract also contained proteoses or peptones, or nitrogen of nonprotein character.

Careful addition of a 16 per cent aqueous solution of trichloroacetic acid to 10 cubic centimeters of the fresh extract precipitated all but a negligible quantity of the nitrogen.

BEHAVIOR OF ARACHIN TOWARD TRICHLOROACETIC ACID, TANNIC ACID, AND TUNGSTIC ACID

Five grams of pure, freshly prepared arachin was dissolved in 10 per cent sodium chloride solution, and the solution diluted in a volumetric flask with water to 100 c. c. Four 5 c. c. portions of the solution were measured out. One portion was used for the determination of total nitrogen, the second was treated with 15 per cent trichloroacetic acid until no further precipitation occurred, the third was similarly treated with an aqueous 10 per cent solution of tannic acid, and the fourth with a mixture of equal volumes of sodium tungstate and two-thirds normal sulphuric acid. These reagents are commonly used as specific precipitants for intact protein. The resulting precipitates were removed by filtration and washed with 10 per cent sodium chloride solution containing a few drops of the precipitating reagent. Nitrogen was determined in the combined filtrate and washings. The results given in Table 3 show that the three reagents

⁵ The term "intact protein molecules" is used to refer to whole, original protein molecules precipitable by trichloroacetic acid and tungstic acid, as distinguished from smaller molecules, such as proteoses and peptones that may occur as products of the first stages of degradation of parent protein molecules.

precipitated practically all the intact protein. Five cubic centimeters of the arachin solution contained 0.02527 gm. of nitrogen.

TABLE 3.—Quantity of arachin precipitated out of 10 per cent sodium chloride solution by various protein precipitants

[The solution originally contained 0.02527 gm. of nitrogen]

Precipitant	Nitrogen left in the filtrate		Percentage of arachin precipitated
	Grams	Percentage of total nitrogen	
Trichloroacetic acid.....	0.00028	1.11	98.99
Tannic acid.....	.0002	.79	99.21
Tungstic acid.....	.0003	1.18	98.82

In order to find out whether any free amino acids might also be precipitated by these reagents along with the protein, precipitation tests were made with arachin in the presence of a mixture of amino acids. The amino acid mixture was prepared by boiling 50 gm. of the α -globulin of tomato seed with a mixture of equal volumes of concentrated hydrochloric acid and water until the hydrolysis was completed, as shown by a negative biuret test. Most of the free hydrochloric acid was removed by concentrating the hydrolysate under reduced pressure to a thick sirup. The residue was dissolved in water, and the solution again concentrated. The final residue was dissolved in water and neutralized with sodium hydroxide.

After the solution was slightly acidified with hydrochloric acid, an equal volume of 95 per cent alcohol was added, followed by the addition of a 10 per cent suspension of calcium hydroxide until all the humin was thrown down, and the solution remained clear. The ammonia was then removed by distilling the mixture under reduced pressure until about half the volume had passed over. The concentrated solution of amino acids was slightly acidified and warmed with charcoal on the steam bath for an hour. The mixture was filtered, and the filtrate diluted with water to 500 c. c.

Portions containing 5 c. c. of the arachin solution and 0.5 c. c. of the amino acid solution (which also contained 10 per cent sodium chloride) were treated with trichloroacetic acid, tannic acid, and tungstic acid in the manner described. The precipitates were filtered off and washed, and nitrogen was determined in the filtrates. As shown in Table 4, practically none of the amino acid nitrogen was precipitated with the arachin.

TABLE 4.—Precipitation of arachin nitrogen from 10 per cent sodium chloride solution by various protein precipitants, in the presence of amino acids

[Nitrogen in 5 c. c. of arachin solution, 0.0171 gm.; nitrogen in 0.5 c. c. of amino acid mixture, 0.0053 gm.]

Precipitant	Nitrogen left in filtrate	Nitrogen in precipitate ^a
	Grams	Grams
Trichloroacetic acid.....	0.0052	0.0172
Tannic acid.....	.0057	.0187
Tungstic acid.....	.0054	.0170

^a By difference.

The precipitation of arachin from a solution containing peptone was also studied. A 10 per cent sodium chloride solution of a commercial peptone was prepared. To portions of a mixture containing 5 c. c. of arachin solution and 0.5 c. c. of the peptone solution, trichloroacetic acid, tannic acid, and tungstic acid were separately added in the manner described. The precipitates were washed, and nitrogen was determined in the filtrates. As shown in Table 5, trichloroacetic acid precipitated all the arachin but none of the peptone. In addition to the arachin nitrogen, 39.13 per cent of the peptone nitrogen was precipitated by tannic acid and 44.66 per cent by the tungstic acid.

TABLE 5.—*Precipitation of arachin nitrogen from a 10 per cent sodium chloride solution by various protein precipitants, in the presence of peptone*

[Nitrogen in 5 c. c. of arachin solution, 0.0171 gm.; nitrogen in 0.5 c. c. of peptone solution, 0.00253 gm.]

Precipitant	Nitrogen left in filtrate
Trichloroacetic acid.....	Grams 0.00253
Tannic acid.....	.00154
Tungstic acid.....	.00140

QUANTITATIVE ESTIMATION OF ARACHIN AND CONARACHIN

The percentage of arachin was determined by three different methods which gave closely agreeing results.

ESTIMATION OF ARACHIN BY PRECIPITATION WITH AMMONIUM SULPHATE

The wide difference in the precipitation limits of arachin and conarachin with ammonium sulphate affords an effective means for their separation.

For the quantitative estimation of arachin 500 c. c. of the fresh, clear filtered sodium chloride extract of peanut meal was placed in a centrifuge bottle, and enough ammonium sulphate added to make the solution 40 per cent saturated. After standing for several hours the mixture was centrifuged. The arachin settled as a viscous, compact layer on the bottom, from which the clear supernatant liquid could be readily decanted. The arachin was again redissolved in 10 per cent sodium chloride solution and reprecipitated with ammonium sulphate in the manner just described. For further purification it was again redissolved in as little 10 per cent salt solution as possible and finally precipitated by dilution with 10 volumes of distilled water. After the whole was centrifuged, the precipitate was dried by first suspending it for 24 hours each in 95 per cent alcohol, and then in absolute alcohol, and finally in absolute ether. The white material was then allowed to stand in a vacuum dessicator over sulphuric acid. Triplicate determinations made in this manner yielded 2.85, 2.84, and 3 gm. of arachin, respectively. Since these quantities were obtained from 500 c. c. aliquots of 2 liters of extract from 50 gm. of meal, they represent, respectively, 22.8, 22.7, and 24 per cent, or an average of 23.2 per cent of the oil-free peanut meal. Even with the exercise of greatest care, some losses occurred during the purification of the arachin. Consequently these figures are somewhat low.

ESTIMATION OF ARACHIN AND CONARACHIN

COAGULATION OF CONARACHIN

Inasmuch as it had been found that, with the exception of a negligible quantity, all the nitrogen in the fresh salt extract of the meal was precipitable with trichloroacetic acid, and that practically all the nitrogen in the extract represented arachin and conarachin, for the estimation of arachin and conarachin advantage was taken of the wide range in the temperatures at which these two proteins coagulate. Ten cubic centimeters of the extract was slowly heated to 85° C. The coagulum was removed on a folded filter and well washed with 10 per cent sodium chloride solution. Determination of nitrogen in the filtrate from the coagulated conarachin gave a value equivalent to 2.3 gm. of arachin nitrogen calculated for the 2 liters of extract from 50 gm. of meal. Since arachin contains 18.29 per cent of nitrogen, the foregoing quantities of nitrogen corresponds to 12.5 gm. of arachin, or 25 per cent of the peanut meal.

Subtracting the arachin nitrogen from the total nitrogen in the extract gave a value corresponding to 9.4 per cent of conarachin.

ESTIMATION OF ARACHIN AND CONARACHIN BY MEANS OF THE REFRACTOMETER

These determinations were based on the method described by Robertson (?), who showed that the change in refractive indices of aqueous solutions owing to the introduction of protein is proportional to the concentration of the protein, and conforms to the formula

$$n - n' = ac$$

where n is the refractive index of the protein solution, n' that of the solvent, c the percentage of protein in the solution, and a a constant characteristic for the protein. Reiss (5) had previously shown that the refractive indices of closely related globulins did not differ sufficiently to distinguish between them. In the determinations reported here it was accordingly assumed that the difference in the refractive indices of arachin and conarachin was so small as to have no material significance in the results.

The measurements were made with a Carl Zeiss refractometer, and a Palo light was used. The 10 per cent sodium chloride extract of the meal used was found to have a refractive index of 1.3530, and that of the pure salt solution, 1.3510. The extract was heated carefully to 85° C., and the coagulated conarachin settled by centrifugalization. The refractive index of the clear supernatant liquid was found to be 1.3525. The percentages of arachin and conarachin were calculated as follows:

$$1.3530 - 1.3510 = 0.0020, \text{ refraction due to arachin and conarachin}$$

$$1.3525 - 1.3510 = 0.0015, \text{ refraction due to arachin}$$

$$0.0005, \text{ refraction due to conarachin}$$

Substituting these values in the formula

$$n - n' = ac. \quad (a = 0.00236)$$

$$\frac{0.00150}{0.00236} = 0.635 \text{ per cent, or } 0.635 \text{ gm. arachin in } 100 \text{ c. c. of extract.}$$

$$\frac{0.0005}{0.00236} = 0.2118 \text{ per cent, or } 0.2118 \text{ gm. conarachin in } 100 \text{ c. c. of extract.}$$

Two liters, or the total extract from 50 gm. of meal, would, therefore, contain 12.70 gm. of arachin and 4.236 gm. of conarachin, or 25.4 per cent and 8.4 per cent, respectively, of the meal.

ESTIMATION OF CONARACHIN BY PRECIPITATION WITH TRICHLORACETIC ACID

One hundred cubic centimeters of the extract was made 40 per cent saturated with ammonium sulphate, and the precipitated arachin removed by filtration. Conarachin was precipitated from the filtrate by addition of trichloroacetic acid. The precipitate was filtered off and washed repeatedly with small portions of dilute trichloroacetic acid until all the ammonium salts were removed. The precipitate of conarachin contained 0.03933 gm. of nitrogen. The original 2 liters of extract from 50 gm. of meal would, therefore, contain 0.7665 gm. of nitrogen, equivalent to 8.4 per cent of conarachin in the oil-free meal.

The results of the various determinations of arachin and conarachin are summarized in Table 6. The percentages obtained by the various methods are in fairly close agreement.

TABLE 6.—*Percentages of arachin and conarachin in oil-free peanut meal as determined by various methods*

Method of estimation	Arachin	Conarachin
	<i>Per cent</i>	<i>Per cent</i>
Isolation by fractional precipitation with ammonium sulphate *	22.8	
	22.0	
	24.0	
Coagulation of conarachin and estimation of arachin in the filtrate	25.0	9.4
Determination by means of the refractometer	25.8	8.4
Precipitation with trichloroacetic acid		8.4
Average	23.8	8.7

* The figures obtained by this method are somewhat too low on account of losses incident to the isolation and purification of the arachin. The quantity of arachin in the meal is nearer 25 per cent than 23.8 per cent, the average of all the determinations.

EXAMINATION OF PEANUT MEAL FOR PROLAMIN

Fifty grams of the meal was stirred for four hours with approximately 1 liter of 65 per cent alcohol. Only 1.5 per cent of the total nitrogen in the meal was extracted. The filtered alcoholic extract was evaporated to dryness. The residue consisted chiefly of a hard, yellow material which had a sweet taste, and gave a strong carbohydrate test with Molisch reagent. It contained 0.5 per cent nitrogen. From these results it was concluded that peanuts contain but a negligible quantity, if any, of the protein of the prolamins class.

EXAMINATION FOR GLUTELIN

A quantity of the peanut meal was exhaustively extracted with 10 per cent sodium chloride solution to remove the globulins, and the residue was then stirred for eight hours with 0.2 per cent sodium hydroxide solution. The alkali extracted 4 per cent of the total nitrogen of the meal. On acidification with acetic acid the extract yielded a brown substance which contained 9.52 per cent nitrogen. The character and properties of this material were not characteristic of a glutelin. This portion represents 11 to 12 per cent of the total nitrogen of the meal.

SUMMARY

Oil-free meal obtained by ether extraction of finely ground shelled Virginia peanuts was found to contain 7.36 per cent nitrogen, equivalent to 40.48 per cent crude protein ($N \times 5.5$).

Ten per cent sodium chloride solution extracted from the oil-free meal 6.41 per cent nitrogen, or 35.27 per cent crude protein.

Examination of the peanut meal showed the presence of only two globulins, arachin and conarachin, present to the extent of approximately 25 per cent and 8 per cent, respectively, of the oil-free meal.

Arachin in 10 per cent sodium chloride solution does not coagulate even at boiling temperature of the saline solution. It has the specific rotation, $[\alpha]_D^{20} = -39.5$, and precipitates completely from its sodium

chloride solution at 40 per cent saturation with ammonium sulphate.

Conarachin coagulates at 80° C. It has a specific rotation, $[\alpha]_D^{20} = -42.7^\circ$, and is precipitated at 85 per cent saturation with ammonium sulphate.

Arachin in 10 per cent sodium chloride solution was practically completely precipitated by trichloroacetic, tannic, or tungstic acids. Amino acids, if present, did not come down with the arachin. In the presence of peptone trichloroacetic acid precipitated all of the arachin but none of the peptone; tannic acid precipitated 39.13, and tungstic acid 44.66 per cent of the peptone nitrogen in addition to the arachin when both the latter were present in 10 per cent sodium chloride solution.

No evidence was obtained showing the presence in peanuts of significant quantities of albumin, prolamin, or glutelin.

LITERATURE CITED

- (1) JOHNS, C. O., and JONES, D. B.
1916. THE PROTEINS OF THE PEANUT, *ARACHIS HYPOGÆA*. I. THE GLOBULINS ARACHIN AND CONARACHIN. *Jour. Biol. Chem.* 28: 77-87.
- (2) ——— and JONES, D. B.
1917. THE PROTEINS OF THE PEANUT, *ARACHIS HYPOGÆA*. II. THE DISTRIBUTION OF THE BASIC NITROGEN IN THE GLOBULINS ARACHIN AND CONARACHIN. *Jour. Biol. Chem.* 30: 33-38.
- (3) ——— and JONES, D. B.
1918. THE PROTEINS OF THE PEANUT, *ARACHIS HYPOGÆA*. III. THE HYDROLYSIS OF ARACHIN. *Jour. Biol. Chem.* 36: 491-500.
- (4) JONES, D. B., GERSDORFF, C. E. F., and MOELLER, O.
1924. THE TRYPTOPHANE AND CYSTINE CONTENT OF VARIOUS PROTEINS. *Jour. Biol. Chem.* 62: 183-195.
- (5) REISS, E.
1904. DER BRECHUNGSKOEFFIZIENT DER EIWEISSKÖRPER DES BLUTSERUMS. *Beitr. Chem. Physiol. u. Path.* 4: 150-154.
- (6) RITTHAUSEN, H.
1880. UEBER DIE EIWEISSKÖRPER VERSCHIEDENER OEELSAMEN. *Pfluger's Arch. Physiol.* 21: 81-104.
- (7) ROBERTSON, T. B.
1910. ON THE REFRACTIVE INDICES OF CERTAIN PROTEINS. III. SERUM GLOBULIN. *Jour. Biol. Chem.* 8: 441-448.

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NUCLEAR DIVISIONS IN THE POLLEN MOTHER CELLS OF TRITICUM, AEGILOPS, AND SECALE AND THEIR HYBRIDS¹

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INTRODUCTION

The phylogenesis of wheat has been a productive field of speculation and investigation, particularly since the early part of the nineteenth century. The various theories proposed to explain the origin of wheats have been based principally on the results of taxonomic studies. According to one of the views all of the species of *Triticum* have originated from the wild prototype *Triticum aegilopoides*. Another view is that the wheats are polyphyletic and that *T. aegilopoides* and *T. dicoccoides* are the wild prototypes of the einkorn and emmer groups, respectively, while the prototype of the *vulgare* group is still unknown. A more recent view derives the species of the latter group (*vulgare*) by hybridization of members of the emmer wheat groups with *Aegilops cylindrica* and *A. ovata*. The ease with which it is possible to cross the three genera *Triticum*, *Secale*, and *Aegilops* suggests a relationship between them which upon further investigation may definitely establish the origin of the wheats. The development of cytology has afforded the opportunity for an analysis of the problem from a new angle. A recent review of the literature on this subject by Bleier (6)² indicates the extent to which cytology has been recognized in analyzing the genetical material accumulated by investigators interested in this problem.

The results of cytological studies of the genera *Triticum*, *Secale*, *Aegilops*, and some of their hybrids are presented in this paper.

MATERIAL AND METHODS

The species of *Aegilops*, *Secale*, and *Triticum* used in making the hybrids herein reported were obtained from various sources. Two varieties of *A. crassa*, namely, *rubiginosa* and *rufescens*, were obtained from C. O. Johnston, engaged in cooperative leaf-rust investigations of the United States Department of Agriculture and the Kansas Agricultural Experiment Station at Manhattan, Kans., who received them from the Russian Bureau of Applied Botany. The remainder of the species of *Aegilops* were obtained through the Office of Foreign Plant Introduction, United States Department of Agriculture. With

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² Reference is made by number (italics) to "Literature cited," p. 717.

the exception of *A. crassa*, the species of *Aegilops* studied, or used in making hybrids, agree with the descriptions of those given by Ascherson and Graebner (2).

The material of *Secale montanum* used in these studies was obtained from E. B. Mains, engaged in cooperative leaf-rust investigations of the United States Department of Agriculture and Purdue University Agricultural Experiment Station, at La Fayette, Ind. The forms of *Triticum* and two varieties of *S. cereale* used are from the collection of the Office of Cereal Crops and Diseases.

All the crosses were made by the junior writer. The F_1 plants and parents were grown in the greenhouses at Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. All of the F_1 plants were grown side by side in the same greenhouse under as nearly identical conditions as possible, thus reducing to a minimum the chance of one hybrid being more favorably situated than another. This procedure seemed a desirable precaution against a possible unfair comparison, particularly in those individuals whose loosely paired chromosomes may be subject to change in behavior under different environments.

Cytological studies were made on the following F_1 hybrids:

Female		Male
<i>T. dicoccoides</i> var. <i>aaronsohni</i>	×	<i>T. monococcum</i> var. <i>flavescens</i> .
<i>T. turgidum</i> var. C. I. 7821.....	×	<i>T. monococcum</i> var. <i>flavescens</i> .
<i>T. polonicum</i> var. C. I. 7070.....	×	<i>T. monococcum</i> var. <i>flavescens</i> .
<i>T. compactum</i> var. Dale Gloria.....	×	<i>T. monococcum</i> var. <i>flavescens</i> .
<i>T. vulgare</i> var. C. I. 7843.....	×	<i>T. monococcum</i> var. <i>flavescens</i> .
<i>T. vulgare</i> var. C. I. 7959.....	×	<i>T. monococcum</i> var. <i>flavescens</i> .
<i>T. spelta</i> var. Alstrom.....	×	<i>T. monococcum</i> var. <i>flavescens</i> .
<i>T. dicoccoides</i> var. <i>aaronsohni</i>	×	<i>S. montanum</i> .
<i>T. vulgare</i> var. Fulhio.....	×	<i>S. montanum</i> .
<i>T. vulgare</i> var. Dawson × Kanred.....	×	<i>S. montanum</i> .
<i>T. spelta</i> var. Alstrom.....	×	<i>S. montanum</i> .
<i>T. vulgare</i> var. Gipsy.....	×	<i>S. cereale</i> .
<i>S. cereale</i>	×	<i>S. montanum</i> .
<i>A. cylindrica</i> var. <i>rubiginosa</i>	×	<i>T. dicoccoides</i> var. <i>aaronsohni</i> .
<i>A. cylindrica</i> var. <i>rubiginosa</i>	×	<i>T. polonicum</i> var. C. I. 7842.
<i>A. crassa</i> var. <i>rubiginosa</i>	×	<i>T. dicoccoides</i> var. <i>aaronsohni</i> .
<i>A. crassa</i> var. <i>rufescens</i>	×	<i>T. dicoccum</i> var. Black Winter.
<i>A. crassa</i> var. <i>rufescens</i>	×	<i>T. dicoccum</i> var. Khapli.
<i>A. crassa</i> var. <i>rufescens</i>	×	<i>T. durum</i> var. C. I. 7789.
<i>A. crassa</i> var. <i>rufescens</i>	×	<i>T. turgidum</i> var. Alaska.
<i>A. crassa</i> var. <i>rufescens</i>	×	<i>T. polonicum</i> var. C. I. 7842.
<i>A. crassa</i> var. <i>rufescens</i>	×	<i>T. compactum</i> var. Dale Gloria.
<i>A. crassa</i> var. <i>rufescens</i>	×	<i>T. vulgare</i> var. Purple Straw.
<i>A. crassa</i> var. <i>rubiginosa</i>	×	<i>T. spelta</i> var. Alstrom.
<i>A. triuncialis</i>	×	<i>A. ovata</i> .

The F_3 plants of the cross *A. ovata* × *T. dicoccum* var. Black Winter^F studied cytologically were grown under conditions similar to those of the F_1 plants referred to above. Several heads of each of the F_1 , F_2 , and F_3 plants of this cross were carefully inclosed in glassine bags or isolated before pollination occurred, thus preventing any opportunity for contamination by cross-pollination.

After making a preliminary examination of the pollen mother cells to determine the proper stage of development, the junior writer collected the young heads of the various species and hybrids and put them immediately into vials of acetic absolute (1-3) killing fluid. At the end of 20 to 30 minutes the killing fluid was poured off and the heads were covered with absolute alcohol. This alcohol was later

changed several times, until no odor of acetic acid was detected. The vials were then corked tightly and put away for future study.

The collections were made in the spring of 1927 and studied in the laboratory during the following winter.

The anthers were dissected out and put on a slide with a few drops of aceto-carmine staining fluid. The anthers were then cut open and pressed lightly, forcing the pollen mother cells out into the liquid. The fragments of the anther walls were next removed with the edge of a cover glass, leaving the cells floating in the liquid. The cover glass was placed carefully on the slide to prevent the cells from floating out beyond the edge of the cover glass. The preparation was then allowed to remain in this condition until sufficient liquid had evaporated to permit the cover glass to fit snugly on the slide. When suitable material was obtained, the edges were sealed with paraffin to prevent further evaporation of the liquid. A slide prepared and sealed in this manner often kept satisfactorily for a week or more.

Material was examined with a Leitz $\frac{1}{8}$ -oil-immersion objective and $\times 8$, $\times 10$, and $\times 15$ oculars. Camera-lucida drawings were made of a large number of stages, a continuous series of pictures of the various meiotic phases being obtained when possible. In addition to drawings a large number of photomicrographs were taken with a Leitz microcamera. The magnifications are shown in the legends.

CHROMOSOMES IN *SECALE CEREALE*

The chromosome number for *Secale cereale* was definitely established as 7 by Sakamura (33). Prior to this time Nemec (25) reported 12 as the diploid number, and Spillman (43) stated that the haploid number is 6, "I believe." Nakao (24), on the other hand, found 8 as the reduced number.

The work of recent investigators, as shown in Table 1, has substantiated Sakamura's determination of 7 as the chromosome number for this species. Stolze (44) found 7 to be the reduced chromosome number for *Secale montanum* also. Several of these investigators, however, found plants of *S. cereale* with 7 + 1 as the haploid number. This number is likely to arise from the diploid species when non-disjunction causes an unequal distribution of the chromosomes in the reduction phases. Under such circumstances the pollen and egg cells will have extra chromosomes.

TABLE 1.—Chromosome numbers of *Secale cereale* and *S. montanum* as determined by the investigators named

[Diploid chromosome numbers in figure columns are shown in italics; all other numbers are haploid chromosomes]

Investigator	<i>S. cereale</i>	<i>S. montanum</i>	Investigator	<i>S. cereale</i>	<i>S. montanum</i>
Nemec, 1910 (25).....	12	-----	Stolze, 1925 (44).....	14, 7	{ 6
Spillman, 1911 (43).....	6	-----			{ 14, 7
Nakao, 1911 (24).....	8	-----	Belling, 1925 (5).....	{ 7	-----
Sakamura, 1918 (33).....	14, 7	-----	Aase and Powers, 1926 (1).....	8	-----
Ferrand, 1923 (9).....	7	-----	Thompson, 1926 (46).....	7	-----
Kihara, 1924 (19).....	{ 14, 7	-----	Present writers.....	7	-----
Nikolaewa, 1924 (28).....	{ 16, 8	-----			
Gotoh, 1924 (13).....	{ 7	-----			
	8	-----			

In the present study it was found that *Secale cereale*, var. Abruzzes, has 7 as its reduced chromosome number. Figure 1, A, B, and C,

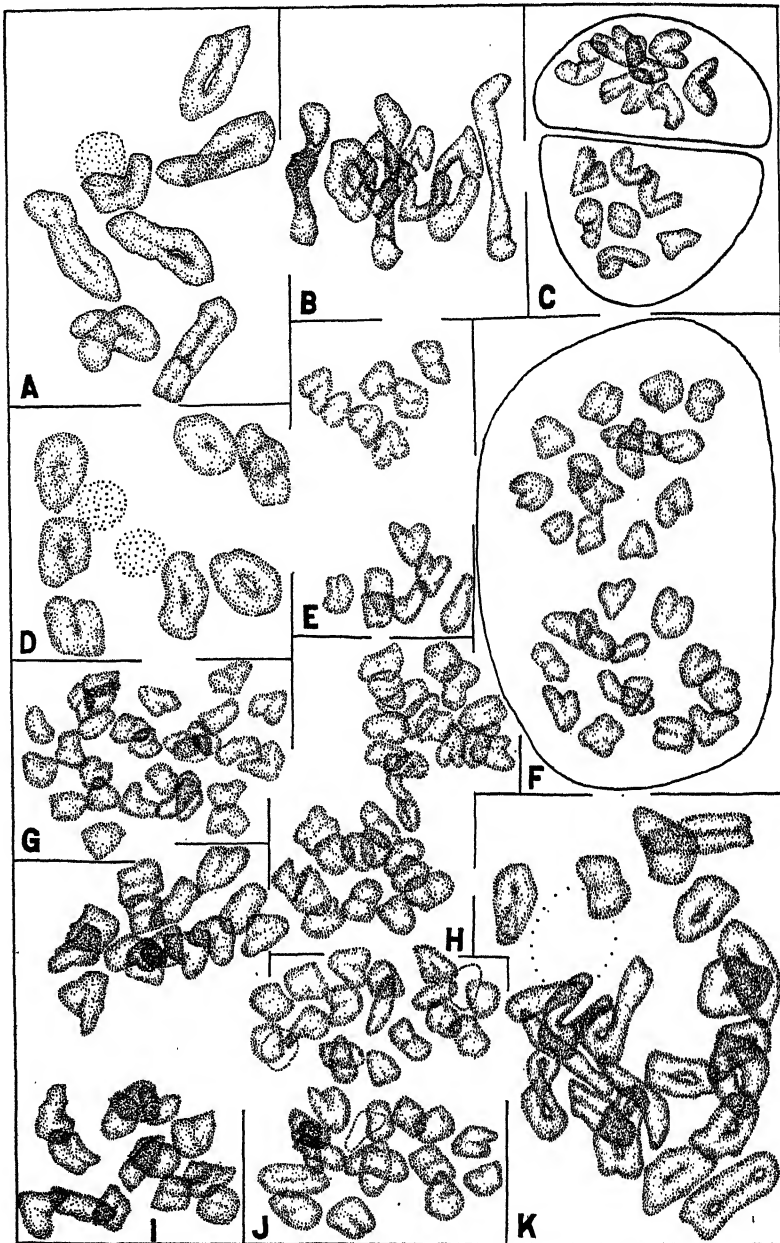


FIGURE 1.—Chromosomes in *Secale* and *Triticum* species. $\times 2,000$: A, Diakinesis in *S. cereale* L.; B, heterotypic metaphase in *S. cereale* L., showing the character, position, and shape of the chromosomes at this phase; C, homotypic metaphase in *S. cereale* L.; D, diakinesis in *T. monococcum* L.; E, late heterotypic anaphase in *T. monococcum* L.; F, late heterotypic anaphase in *T. dicoccoides* Koke; G, heterotypic anaphase in a regular form of *T. polonicum* L.; H, heterotypic anaphase in *T. dicoccum* Schrk.; I, heterotypic anaphase in *T. turgidum* L.; J, heterotypic anaphase of an apparently fixed segregate of *T. vulgare* \times *T. polonicum*, showing 33(-36?) chromosomes; K, diakinesis in *T. compactum* Host.

shows three reduction phases. The chromosomes are all paired at diakinesis and are distributed regularly in practically every figure studied.

Considerable attention was given to the shape of the individual chromosomes as they appeared on the heterotypic metaphase plate. Figure 2 is a diagrammatic representation of the 7 chromosomes at this phase. Three of the chromosomes, A, B, and C, have only slightly subterminal points of fiber attachment. At the late metaphase the halves of these three chromosomes are stretched apart and seem much longer than chromosomes D, E, F, and G. The latter have almost central points of fiber attachment, vary in shape from the open C type to the closed double V, and appear much shorter than chromosomes A, B, and C. Figure 1, B, shows the 7 chromosomes in a characteristic position. The two halves of the 3 chromosomes with subterminal fiber attachment appear as rods or J's and are easily distinguished from the other 4 chromosomes. In the early anaphase

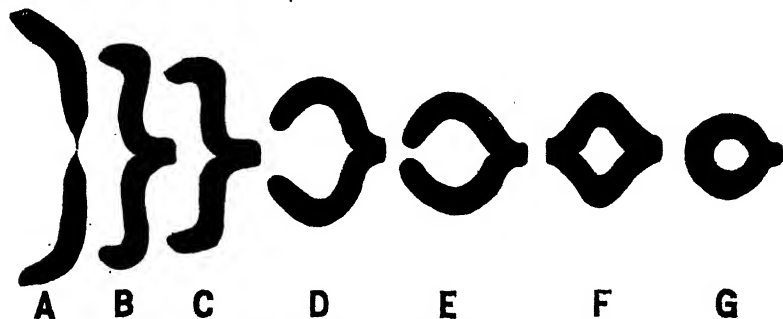


FIGURE 2.—Characteristic shapes of individual chromosomes in rye

stage all halves of the chromosomes D, E, F, and G are V-shaped and show only slight differences in the points of fiber attachment. Gotoh (18) presents similar pictures for *Secale* chromosomes. The differences between the shapes of the chromosomes illustrated by Gotoh and those pictured by the writers probably are due to the variable positions assumed by the chromosomes while under tension during division.

CHROMOSOMES IN F_1 HYBRIDS OF *SECALE CEREALE* × *S. MONTANUM*

The haploid chromosome number of *Secale cereale* is 7, with few exceptions. Stolze (44) has found the same number for *S. montanum*. The F_1 hybrid which provided cytological material for the writers' study has 7 chromosomes as the reduced number, as was anticipated.

The generally observed behavior of the chromosomes in the meiotic phases of the developing pollen mother cell of the F_1 hybrid studied by the writers was regular (fig. 3, A), giving tetrads with 7 chromosomes in each cell. Occasionally abnormal chromosome behavior was observed in diakinesis and in the heterotypic anaphase. In diakinesis a few unpaired chromosomes may be observed distributed among the bivalents. In the heterotypic anaphases six bivalent and two univalent chromosomes were sometimes seen. Under such conditions the following variable distribution of the univalent chromosomes may occur: (1) Both univalent chromosomes pass undivided to one pole

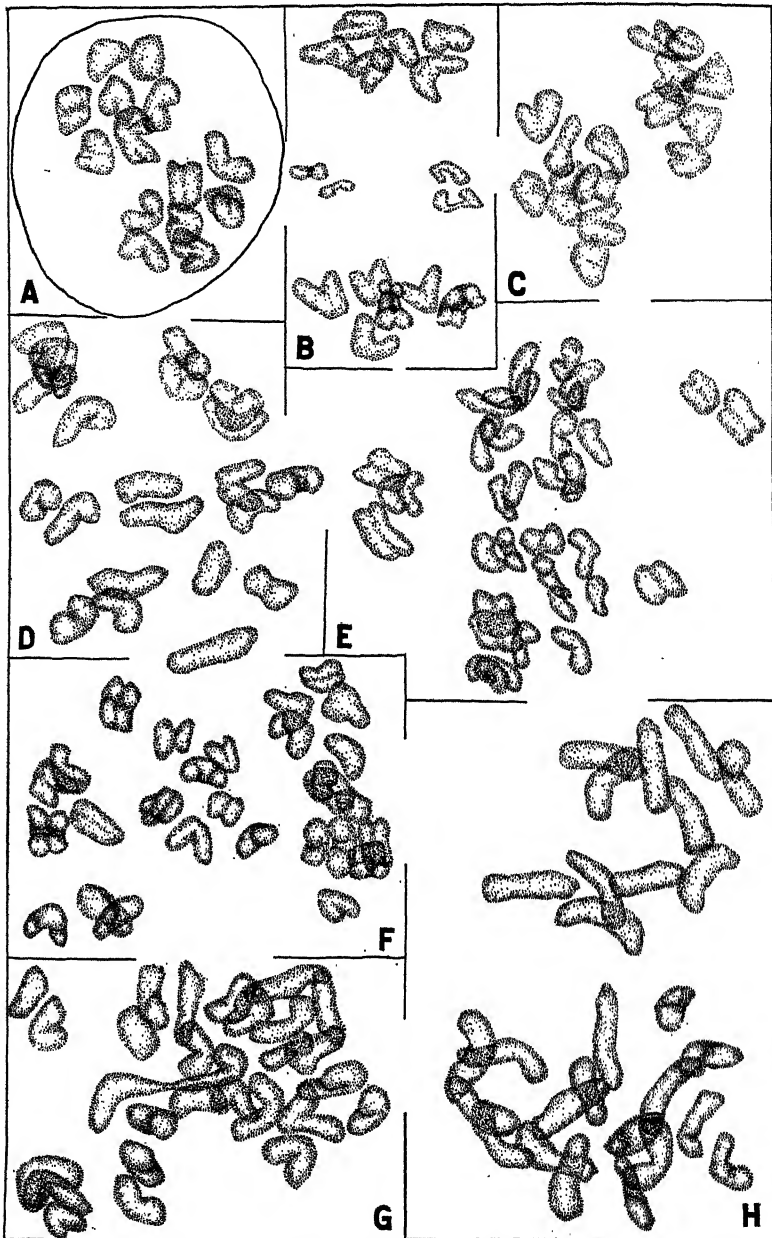


FIGURE 3.—Chromosomes in *Secale* \times *Secale* and *Secale* \times *Triticum* hybrids. $\times 2,000$; A, Late heterotypic anaphase in *S. cereale* \times *S. montanum*; B, late heterotypic anaphase in *S. cereale* \times *S. montanum*, showing 6 chromosomes at each pole and 2 split univalents on the spindle; C, heterotypic anaphase in *S. cereale* \times *S. montanum*, showing 6 chromosomes going to one pole and 8 to the other; D, heterotypic prophase in *T. dicoccoides* \times *S. montanum*, showing 21 univalent chromosomes; E, heterotypic metaphase in *T. dicoccoides* \times *S. montanum*, showing a few chromosomes at each pole and splitting univalents on the equatorial plate; F, heterotypic anaphase in *T. vulgare* \times *S. montanum*, showing a few chromosomes at the equatorial plate region; G, heterotypic metaphase in *T. vulgare* \times *S. montanum*, showing a bivalent chromosome; H, heterotypic prophase in *T. vulgare* \times *S. cereale*, showing 28 unpaired chromosomes

(fig. 3, C); (2) one chromosome passes to each pole; (3) the two univalents split longitudinally and tardily join the earlier divided chromosomes, giving to each nucleus two types of chromosomes (fig. 3, B); (4) one univalent splits, one half passing to each pole, while the other univalent goes undivided to either pole.

The homotypic division was found to be regular if no halves of univalents were in the chromosome complement. In the homotypic anaphase the halves when present usually lag behind the divided chromosomes and pass at random without further division to the daughter nuclei.

The pollen nucleus of this hybrid usually has seven chromosomes, but the number may be either above or below this basic number, due to such irregularities as those described above.

CHROMOSOMES IN TRITICUM SPECIES

A general study of chromosomes in wheat species was made in conjunction with that of the *Triticum* hybrids.

The chromosome number for most of the *Triticum* species has been definitely established. The determinations of the various investigators are listed in Table 2.

The chromosomes of the various species used by the writers are shown in Figure 1, D-I and K, and Figure 4, D and E. A heterotypic anaphase of *Triticum polonicum*, with 14 chromosomes nearing each pole, is shown in Figure 1, G. This number confirms the studies of other investigators who place *T. polonicum* among the tetraploid species. Another form of wheat (F. S. P. I. No. 56233) that had the long glumes of Polish and the lax head and tough glumes of spelt was also studied. This wheat was received from Portugal with a pedigree showing that it had originated as a cross between *vulgare* and *polonicum* wheats. Judging from morphological characters, it is apparently a fixed segregate from this cross. Figure 1, J, shows a heterotypic anaphase of this odd form with 17 chromosomes at one pole and 16 at the other. In addition to these chromosomes two extra questionable chromosomes may be observed at one pole and one chromosome at the other, giving the reduced number of at least $\frac{33}{2}$ and possibly as

high as 18. A study of the homotypic anaphases shows a few chromosomes irregularly distributed to the poles or extruded into the cytoplasm. As a result of the latter phenomenon many cells in the pollen tetrads have one or more minor nuclei in addition to the major nucleus. It is interesting to find a form apparently fixed with a chromosome number intermediate between that of the two parents. Several crosses were made involving this form, but no hybrids were studied.

Photomicrographs of six meiotic phases of the developing pollen mother cell in *Triticum* species are shown in Plate 1, A-F. They are arranged in sequence and show the regular distribution of chromosomes to the daughter cells. Plate 1, A, is a heterotypic prophase of *T. monococcum* showing seven bivalent chromosomes and the nucleolus. Plate 1, B, shows a typical heterotypic metaphase of *T. vulgare*; C shows an anaphase of *T. vulgare* with a few chromosomes dividing; D shows a heterotypic telophase of *T. vulgare* with two equal-sized chromatin masses at each pole; E shows homotypic telophases of *T. compactum* in which the chromosomes have divided and moved to the poles in a regular manner; F is a typical tetrad of *T. spelta*.

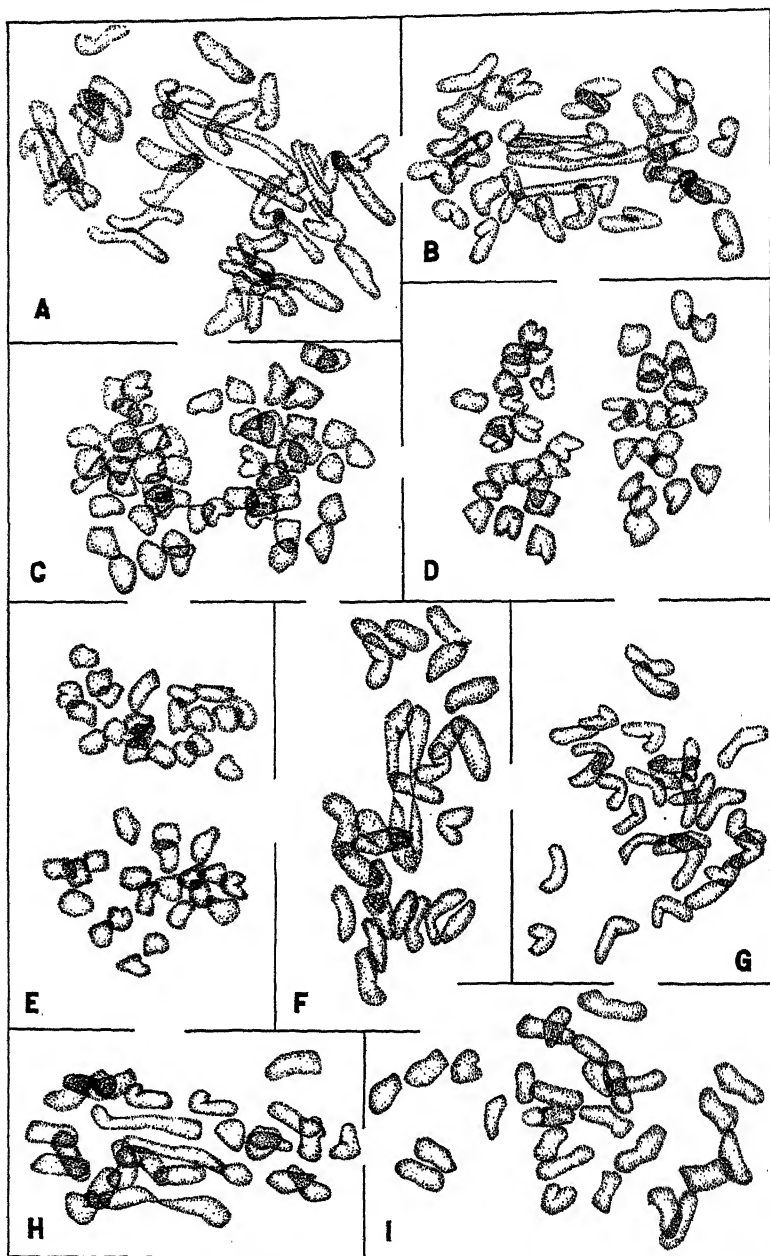


FIGURE 4.—Chromosomes in *Triticum* species and *Aegilops* \times *Triticum* hybrids. $\times 2,000$: A, Heterotypic metaphase in *A. crassa* \times *T. dicoccum*; B, heterotypic metaphase in *A. crassa* \times *T. turgidum*; C, heterotypic anaphase in F_2 plant of *A. ovata* \times *T. dicoccum*; D, heterotypic anaphase in *T. spelta* L.; E, heterotypic anaphase in *T. vulgare* Vill.; F, heterotypic metaphase in *A. cylindrica* \times *T. turgidum*; G, heterotypic prophase in *A. cylindrica* \times *T. turgidum*; H, heterotypic metaphase in *A. cylindrica* \times *T. polonicum*; I, heterotypic prophase in *A. cylindrica* \times *T. polonicum*.

TABLE 2.—Chromosome numbers of some *Triticum* species as determined by the investigators named

[Diploid chromosome numbers in figure columns are shown in italics; all other numbers are haploid chromosomes]

Investigator	T. aegeolopoides	T. monococcum	T. thauroudar	T. dicocoides	T. dicococum	T. durum	T. turgidum	T. persicum	T. pyramidalis	T. polonicum	T. vulgare	T. compactum	T. sphaerococcum	T. spelta
Overton, 1888 (29)											16, 8			
Golofinski, 1893 (12)											16, 8			
Koernicke, 1896 (31)											16, 8			
Spillman, 1911 (48)											16, 8			
Wakao, 1912 (5)											16, 8			
Platy, 1912 (5)											16, 8			
Sato, 1918 (45)											16, 8			
Sato, 1918 (45)											16, 8			
Nikolaev, 1921 (86)											16, 8			
Nikolaev, 1921 (86)											16, 8			
Zhurkovsky, 1923 (51)											16, 8			
Watkins, 1924-25 (48)											16, 8			
Winge, 1924 (49)											16, 8			
Kilbara, 1924 (16)											16, 8			
De Mol, 1924 (25)											16, 8			
Stolze, 1925 (44)											16, 8			
Kagawa, 1927 (14, 15), 1928 (16)											16, 8			
Tscherniak and Haler, 1928 (46)											16, 8			
Thompson, 1928 (10)											16, 8			
Flaksberger, 1928 (10)											16, 8			
Perclval, 1928 (32)											16, 8			
Aase and Powers, 1928 (1)											16, 8			
Gaines and Aase, 1928 (11)											16, 8			
Present writers											16, 8			

*40 or more.

*42 to 44.

*44 to 50.

*According to Flaksberger (10), De Mol's *T. dicocoides* should be *T. aegeolopoides*.

CHROMOSOMES IN F_1 HYBRIDS OF TRITICUM

Numerous investigators have studied the chromosomes in interspecific wheat hybrids. Table 3 lists 26 crosses between different species of wheat in which the chromosome number and behavior have been described.

The present study traces the chromosome behavior of certain *Triticum* × *Triticum* hybrids through many of the important meiotic phases of developing pollen mother cells. These include crosses between *T. monococcum* and species of the emmer group resulting in triploid hybrids, and crosses between *T. monococcum* and species of the *vulgare* group resulting in tetraploid hybrids. *T. monococcum* was used as the pollen parent in crosses with both groups of *Triticum*.

TABLE 3.—Summary of cytological work on F_1 interspecific hybrids of *Triticum*

Hybrid combination		Chromosome number of parents		Chromosome pairing	Authority	Year
Female	Male	Female	Male			
<i>T. aegilopoides</i> ...	<i>T. dicoccum</i> ...	7	14	4-7	Kihara (19).....	1924
Do.....	do.....	7	14	-----	Kihara and Nishiyama (20).....	1928
<i>T. monococcum</i> ...	<i>T. Turgidum</i> ...	7	14	(?) 7	Sax (37).....	1922
Do.....	do.....	7	14	3-7	Thompson (15).....	1926
<i>T. dicoccoides</i> ...	<i>T. monococcum</i> ...	14	7	0-6	Present writers.....	-----
<i>T. dicoccum</i> ...	do.....	14	7	4-7	Kihara (19).....	1924
Do.....	do.....	14	7	-----	Kihara and Nishiyama (20).....	1928
Do.....	<i>T. vulgare</i> ...	14	21	14	Sax (38).....	1923
<i>T. durum</i> ...	do.....	14	21	14	Kihara (17, 18, 19).....	1919, 1921, 1924
Do.....	do.....	14	21	14	Sapehin (24).....	1928
Do.....	do.....	14	21	14	Sax (37).....	1922
Do.....	do.....	14	21	-----	Kihara and Nishiyama (20).....	1923
<i>T. turgidum</i> ...	<i>T. monococcum</i> ...	14	7	0-7	Present writers.....	-----
Do.....	<i>T. compactum</i> ...	14	21	14	Kihara (17, 19).....	1919, 1924
Do.....	<i>T. vulgare</i> ...	14	21	14	Watkins (43).....	1924-25
<i>T. polonicum</i> ...	<i>T. monococcum</i> ...	14	7	0-5	Present writers.....	-----
Do.....	<i>T. compactum</i> ...	14	21	14	Kihara (19).....	1924
Do.....	<i>T. spelta</i> ...	14	21	14	Kihara (17, 19).....	1919, 1924
<i>T. vulgare</i> ...	<i>T. monococcum</i> ...	21	7	0-7	Present writers.....	-----
Do.....	<i>T. durum</i> ...	21	14	14	Sax (37).....	1922
Do.....	<i>T. turgidum</i> ...	21	14	14	do.....	1922
<i>T. compactum</i> ...	<i>T. monococcum</i> ...	21	7	0-7	Present writers.....	-----
Do.....	<i>T. durum</i> ...	21	14	14	Sax (37).....	1922
<i>T. spelta</i> ...	<i>T. aegilopoides</i> ...	21	7	-----	Kihara and Nishiyama (20).....	1928
Do.....	<i>T. monococcum</i> ...	21	7	0-5	Melburn and Thompson (22).....	1927
Do.....	do.....	21	7	0-5	Present writers.....	-----

TRIPLOID HYBRIDS

The abundant cytological material available from the cross *Triticum dicoccoides* × *T. monococcum* made it possible to obtain a continuous picture of the chromosome behavior through both reduction divisions. Characteristic prophase showed both univalent and bivalent chromosomes. The two types of chromosomes are distinguished most easily when the latter are on the equatorial plate, for at this time the univalents are scattered through the nuclear region. A count of 9 cells taken at random gave 1 with 6 bivalents and 9 univalents, 4 with 5 bivalents and 11 univalents, 3 with 4 bivalents and 13 univalents, and 1 with 3 bivalents and 15 univalents. These few counts represent

the combinations most commonly observed, though occasionally less pairing than this may be seen. In no cases were cells observed with more than six paired chromosomes. The marked variability in the number of paired and unpaired chromosomes indicates that no definite number of paired chromosomes is characteristic of this hybrid.

Figure 5, A, shows a heterotypic metaphase, if this phase can be said to exist, with 5 bivalent chromosomes on the plate and 11 univalent chromosomes scattered through the spindle region. In B, which shows a later phase, the bivalents are moving toward the poles and have joined a few of the univalents that failed to move to the equatorial plate. Four univalents are shown just prior to splitting longitudinally. A later stage is shown in D, in which the late-dividing univalents are moving in two groups to the poles. C shows a late anaphase with four groups of chromosomes in their characteristic positions. They are frequently seen arranged in this manner prior to the formation of the two daughter nuclei.

Pollen mother cells undergoing the second reduction division usually appear abnormal, due to the presence of halves of univalent chromosomes in each chromosome group. The halves of bivalents and the undivided univalent chromosomes divide and move to the poles in a regular manner. In the homotypic anaphase the halves of univalent chromosomes that split in the previous division are frequently found lagging on the spindle, as shown in Figure 5, E, and go at random without further division to the poles or lag behind and fail to be included in the daughter nuclei, in which case they form micronuclei. The pollen tetrad shown in Figure 5, F, shows each cell with a major nucleus and from one to several minor nuclei. The number of minor nuclei depends to a large degree on the number of chromosomes that fail to be included in the major nucleus. In 379 tetrads only 2 were found in which all 4 pollen grains were without supernumerary nuclei. In a study of 102 pollen grains 37 had one nucleus only, 49 had a major and a minor nucleus, 13 had a major and 2 minor nuclei, 2 had a major and 3 minor nuclei, and 1 had a major and 4 minor nuclei.

The chromosome behavior in the meiotic phases of the pollen mother-cell development in *Triticum turgidum* \times *T. monococcum* was similar to that described for the previous cross. There seemed to be a somewhat greater tendency for chromosomes to pair in this hybrid. Figure 5, H, shows a metaphase with seven bivalents in the plate region, but other cases were observed showing only unpaired chromosomes. Figure 5, I, shows a later phase, with the divided bivalents at the poles, and nine univalents on the equatorial plate in the process of splitting longitudinally.

The second division shows the same random assortment of the split univalent chromosomes as was described for the previous cross. Many of these chromosomes remain in the cytoplasm and form micronuclei.

The third cross, *Triticum polonicum* \times *T. monococcum*, shows in the early heterotypic phases four or five paired chromosomes as the most common combination. (Fig. 5, G.) The behavior of the univalents is similar to that of the above-described crosses. It is unusual to find at the end of this division any indications that chromosomes have been left in the cytoplasm.

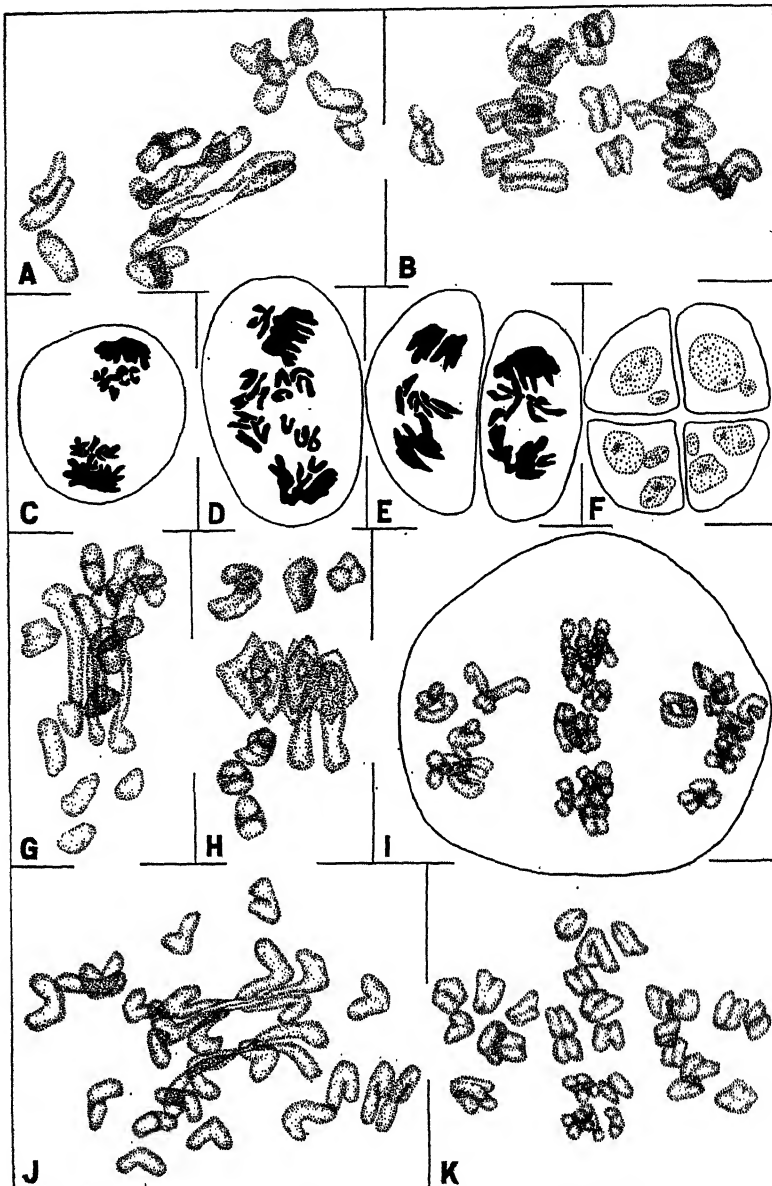


FIGURE 5.—Chromosomes in *Triticum* \times *Triticum* hybrids: A, Heterotypic metaphase in *T. dicoccoides* \times *T. monococcum*; B, heterotypic anaphase in *T. dicoccoides* \times *T. monococcum*; C, late heterotypic anaphase in *T. dicoccoides* \times *T. monococcum*; D, heterotypic anaphase in *T. dicoccoides* \times *T. monococcum*; E, homotypic anaphase in *T. dicoccoides* \times *T. monococcum*; F, tetrad in *T. dicoccoides* \times *T. monococcum*; G, heterotypic metaphase in *T. polonicum* \times *T. monococcum*, showing four bivalents and two univalents in the plate region; H, heterotypic metaphase in *T. turgidum* \times *T. monococcum*; I, late heterotypic anaphase in *T. turgidum* \times *T. monococcum*; J, heterotypic metaphase in *T. compactum* \times *T. monococcum*; K, heterotypic anaphase in *T. compactum* \times *T. monococcum*. A, B, G-K, $\times 2,000$; C-F, $\times 975$.

A characteristic phenomenon of triploid hybrids is the formation of tetrads, each cell of which may contain, besides a major nucleus, one or more minor nuclei. The presence of minor nuclei in the cells of a tetrad suggests that in the heterotypic division some univalent chromosomes split longitudinally and are included in the two daughter nuclei, while in the homotypic division several split univalents fail to be included in the major nuclei and form micronuclei.

Sax (37) was the first to describe the chromosome behavior in developing pollen mother cells of the triploid wheat hybrid *Triticum monococcum* \times *T. turgidum*. Thompson (45) also studied the cross but failed to confirm Sax's report that seven bivalents usually are present in the early heterotypic phase and that the univalents go undivided to the poles in the first division. The results of this study support Thompson's work and show the number of paired chromosomes to be variable, with some univalents splitting longitudinally in the heterotypic division. Kihara (19) described the behavior of chromosomes during the meiotic divisions in the hybrids of two other triploid crosses, *T. aegilopoides* \times *T. dicoccum* and *T. dicoccum* \times *T. monococcum*. His results are similar to those of Thompson and those of the writers herein described except that he found some chromosomes lost in the cytoplasm in the first division. The more recent chromosome study by Kihara and Nishiyama (20) of triploid, tetraploid, and pentaploid wheat hybrids shows the presence of trivalent chromosomes, a combination not observed by the writers in any of the material studied.

TETRAPLOID HYBRIDS

The hybrid *Triticum compactum* \times *T. monococcum* gave abundant material for a study of the important phases in the reduction division of the pollen mother cells. A variable number of univalent and bivalent chromosomes was observed. Figure 5, J, shows an early heterotypic phase with 5 bivalent and 18 univalent chromosomes. Other cells, however, show as high as 7 paired chromosomes, while a few indicate no chromosome pairing. Figure 5, K, shows a later phase with 12 univalent chromosomes on the equatorial plate and groups of chromosomes near each pole composed of the divided bivalents and a few univalents that failed to move into the plate region. Later stages show that the univalent chromosomes which go to the equatorial plate split and the halves move to the poles to join the group that preceded them, thus producing two daughter nuclei each containing two types of chromosomes. Only one case was found in which all the chromatin material was not included in the two-daughter nuclei.

The homotypic division shows, in the anaphase, many of the univalent chromosomes which, having split in the previous division, lag on the spindle. These go at random to the poles and are included with the divided chromosomes in the formation of the daughter nuclei or lag behind and are left in the cytoplasm to form dwarf nuclei.

The chromosome behavior of the third tetraploid cross, *Triticum spelta* \times *T. monococcum*, differs very little from that described for the two preceding crosses. Figure 6, A, shows a cell with 28 univalent chromosomes, while B shows 5 paired and 18 univalent chromosomes. The variation in the number of bivalents observed is within the range of these two illustrations. Many of the univalents split in the first reduction division, and usually all of the chromatin material is in-

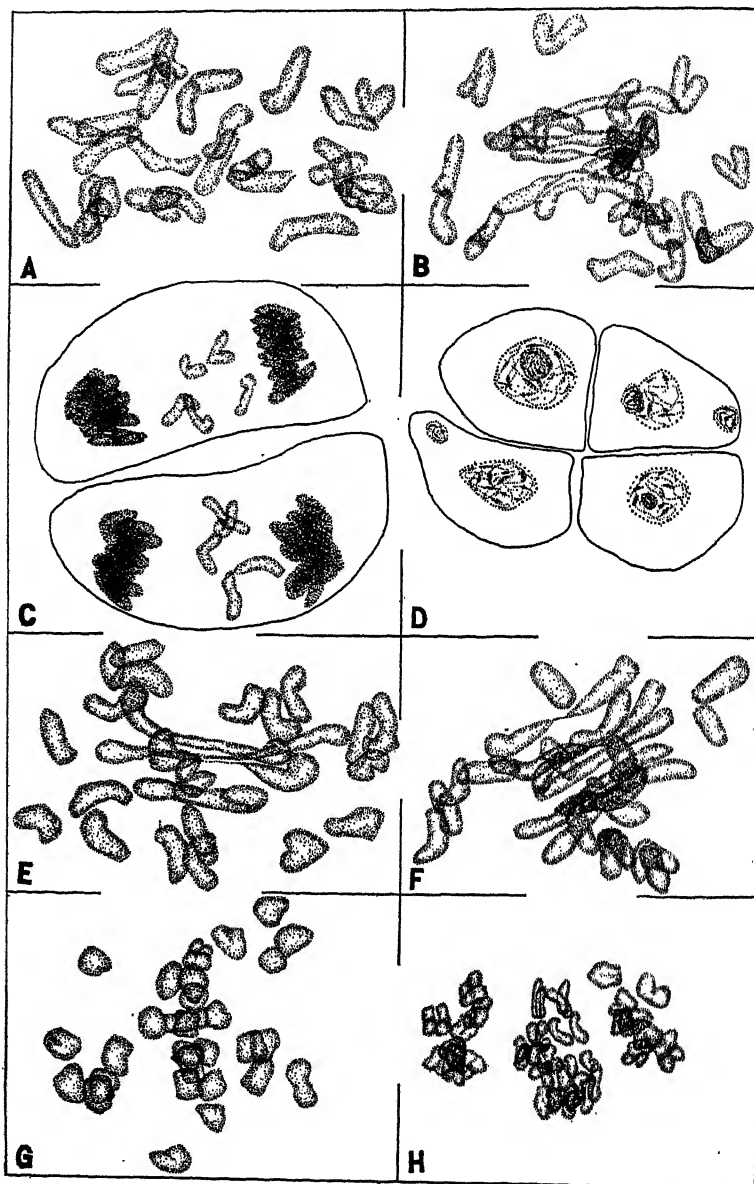


FIGURE 6.—Chromosomes in *Triticum* × *Triticum* hybrids. $\times 2,000$: A, Heterotypic prophase in *T. spelta* × *T. monococcum*; B, heterotypic metaphase in *T. spelta* × *T. monococcum*; C, homotypic anaphase in *T. spelta* × *T. monococcum*; D, tetrad in *T. spelta* × *T. monococcum*; E, F, G, three heterotypic metaphases in *T. vulgare* × *T. monococcum*; H, heterotypic anaphase in *T. vulgare* × *T. monococcum*.

cluded in the two daughter nuclei. C shows a characteristic homotypic anaphase. At this stage several of the halves of univalents produced in the first division are seen lagging on the spindle. In D is shown a characteristic tetrad, each cell having one or two minor nuclei in addition to the major nucleus.

Characteristic heterotypic metaphases and anaphases in the cross *Triticum vulgare* \times *T. monococcum* are shown in Figure 6, E-H. In E are shown 4 bivalent and 20 univalent chromosomes, while in F there are 7 bivalents and 14 univalents. These cells represent about the range of variation observed. In G and H are shown a large number of univalents arranged on the equatorial plate. Later phases show the univalents on the plate splitting longitudinally, the halves joining the earlier divided chromosomes that preceded them to the poles.

The second division is similar to that described for the preceding cross. Tetrads are formed, many individual cells of which are polynucleated.

Melburn and Thompson (22) describe the chromosome behavior in the above tetraploid hybrid. They found 0-5 bivalent and 28-18 univalent chromosomes in the early heterotypic phases. Some of the univalents split in the first reduction division, and the halves were seen lagging on the homotypic anaphase spindle in their random passage to the poles. Some chromosomes were extruded in both divisions. The writers find that only very rarely are chromosomes extruded in the first division, but much extrusion of lagging chromosomes may occur in the second division. In agreement with Melburn and Thompson, the writers find that a majority of the pollen tetrads have only four cells, and that many of these cells may be polynucleated.

PENTAPLOID HYBRIDS

No pentaploid hybrids were studied by the writers, but their studies with triploid and tetraploid hybrids afforded an opportunity to compare the meiotic behavior of their hybrids with that observed by others in pentaploid hybrids.

Kihara (17, 19) was the first to investigate the chromosome behavior of *Triticum* \times *Triticum* hybrids. He found in the early heterotypic phases of pentaploid hybrids 14 bivalent and 7 univalent chromosomes. Many of the bivalent chromosomes were seen to split in the first division, and some of the halves of these split chromosomes were left behind in the cytoplasm in both reduction divisions. Sax (37) reported a very similar behavior with the exception that he found practically no chromosomes left free in the cytoplasm during the first division.

The chromosomes of pentaploid hybrids appear to be distributed to the four daughter cells in pollen formation in a manner similar to that described for triploid and tetraploid *Triticum* \times *Triticum* hybrids.

GENERAL MEIOTIC PHENOMENA IN *TRITICUM* \times *TRITICUM* HYBRIDS

Plate 2 shows a series of photomicrographs intended to give a somewhat continuous picture of the important meiotic phases in the developing pollen mother cells of *Triticum* hybrids.

An early heterotypic prophase of *Triticum compactum* \times *T. monococcum* in which the individual chromosomes can not be distin-

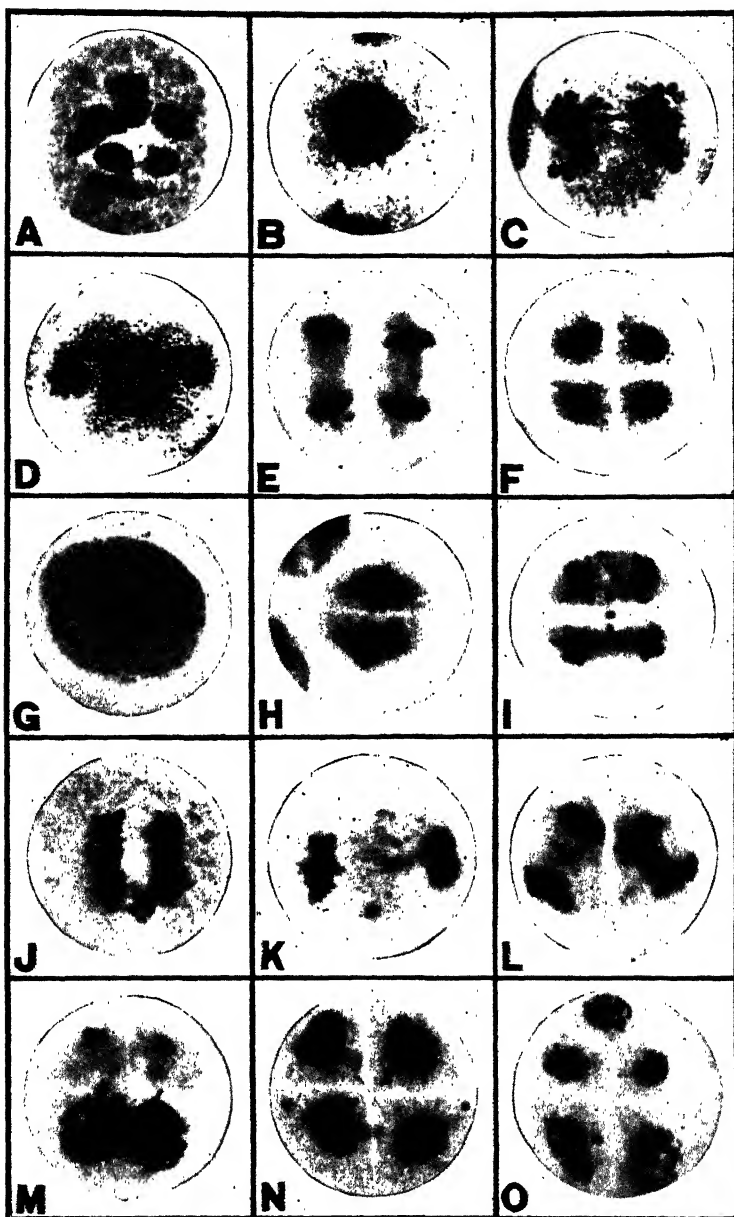
guished is shown in Plate 2, A. B (*T. dicoccoides* \times *T. monococcum*) and C (*T. spelta* \times *T. monococcum*) show typical heterotypic metaphases. The bivalents and a few univalents are at the equatorial plate, and other univalents are scattered through the spindle region. D, E, and F show heterotypic anaphases of *T. turgidum* \times *T. monococcum*, *T. spelta* \times *T. monococcum*, and *T. dicoccoides* \times *T. monococcum*, respectively, in which the groups near each pole are made up of halves of bivalent chromosomes and univalents that failed to move to the equatorial region. In D, other univalents have collected on the plate and are in the process of splitting longitudinally. In E, these chromosomes have split and in F they have moved toward the poles. G shows in *T. turgidum* \times *T. monococcum* two groups of chromosomes at each pole, a condition frequently seen in the early heterotypic telophase. H (*T. compactum* \times *T. monococcum*) and I (*T. turgidum* \times *T. monococcum*) show interkinesis. The appearance of pollen mother cells at this stage gives no indication of the chromosome irregularities that characterize the heterotypic metaphase and anaphase. J (*T. vulgare* \times *T. monococcum*) shows a homotypic metaphase in which most of the chromosomes are at the plates, but a few univalents that split in the previous division are scattered through the spindles. K (*T. spelta* \times *T. monococcum*) and L (*T. dicoccoides* \times *T. monococcum*) are typical homotypic anaphases with chromosomes lagging on the spindle. The micronuclei in the cells of the tetrads of *T. polonicum* \times *T. monococcum*, shown in M and N, are produced by the chromosomes that were seen lagging on the homotypic anaphase spindle and failed to be included in the four major nuclei. O shows a late homotypic telophase of *T. compactum* \times *T. monococcum* in which three instead of two nuclei were produced by the heterotypic division. Such a 3-celled individual will produce a 6-celled tetrad. Tetrads with more than four cells are rare in *Triticum* \times *Triticum* hybrids.

The cytological work on F_1 interspecific hybrids of *Triticum* is summarized in Table 3.

CHROMOSOMES IN F_1 HYBRIDS OF TRITICUM \times SECALE

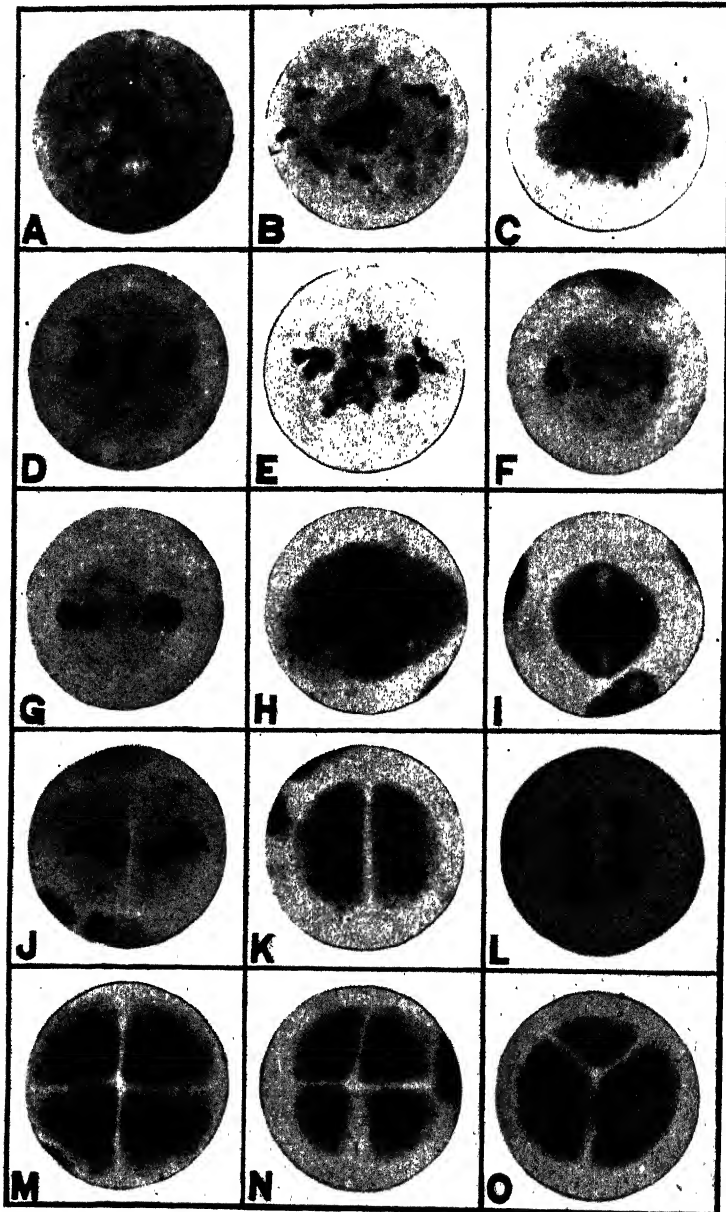
This study includes a triploid and four tetraploid hybrids between the genera *Triticum* and *Secale*.

The hybrid *Triticum dicoccoides* \times *Secale montanum* shows 21 univalent chromosomes scattered through the spindle in the early metaphase stage. Figure 3, D, shows a typical prophase, while E shows a cell with a majority of the chromosomes on the equatorial plate (the nearest approach found to a true metaphase stage). Those on the plate split and move toward the undivided univalents situated near the poles. It is not unusual to find four nuclei, two of which contain only unsplit univalents while the other two have only split univalents. Other cases were found in which split and unsplit univalents were included in a single nucleus on one side of the cell, while on the other side were two nuclei, one of which contained split and the other unsplit univalent chromosomes. Some cases were observed in which a single nucleus containing both split and unsplit chromosomes was situated on each side of the cell. A more abnormal condition was found in which the univalents on the equatorial plate failed to divide, forming a nucleus in this central position, while those at the poles formed other nuclei. Under such conditions three cells were formed. In cases



PHOTOMICROGRAPHS OF MEIOTIC PHASES IN DEVELOPING POLLEN MOTHER CELLS OF TRITICUM SPECIES AND F_1 AEGILOPS \times AEGILOPS AND F_2 AEGILOPS \times TRITICUM HYBRIDS. $\times 875$

A, Diakinesis in *T. monococcum* L.; B, heterotypic metaphase in *T. vulgare* Vill.; C, heterotypic anaphase in *T. vulgare*; D, heterotypic telophase in *T. vulgare*; E, homotypic telophase in *T. compactum* Host; F, tetrad in *T. spelta* L.; G, heterotypic anaphase in *A. triuncialis* \times *A. ovata*; H, homotypic metaphase in *A. triuncialis* \times *A. ovata*; I, homotypic anaphase in *A. triuncialis* \times *A. ovata*; J, heterotypic anaphase in F_2 hybrids of *A. ovata* \times *T. dicoccum*; K, heterotypic telophase in F_2 hybrids of *A. ovata* \times *T. dicoccum*; L, homotypic anaphase in F_2 hybrids of *A. ovata* \times *T. dicoccum*; M, N, O, tetrads in F_2 hybrids of *A. ovata* \times *T. dicoccum*



PHOTOMICROGRAPHS OF MEIOTIC PHASES IN THE DEVELOPING POLLEN MOTHER CELLS OF TRITICUM X TRITICUM HYBRIDS. X 875

A, Early heterotypic prophase in *T. compactum* X *T. monococcum*; B, heterotypic metaphase in *T. dicoccoides* X *T. monococcum*; C, heterotypic metaphase in *T. spelta* X *T. monococcum*; D, heterotypic anaphase in *T. turgidum* X *T. monococcum*; E, heterotypic anaphase in *T. spelta* X *T. monococcum*; F, later heterotypic anaphase in *T. dicoccoides* X *T. monococcum*; G, heterotypic telophase in *T. turgidum* X *T. monococcum*; H, interkinesis in *T. compactum* X *T. monococcum*; I, interkinesis in *T. turgidum* X *T. monococcum*; J, homotypic metaphase in *T. vulgare* X *T. monococcum*; K, homotypic anaphase in *T. spelta* X *T. monococcum*; L, late homotypic anaphase in *T. dicoccoides* X *T. monococcum*; M, N, tetrads in *T. polonicum* X *T. monococcum*; O, an unusual homotypic telophase in *T. compactum* X *T. monococcum*.

where all the univalents go at random to the two poles two cells are formed, each containing a nucleus. The nuclei are equal in size if approximately equal numbers of chromosomes go to each pole, but frequently they are quite unequal when a greater number of chromosomes go to one pole than to the other.

Such erratic behavior of chromosomes in the first division give many types of irregularities in the second division and leads to the production of a variety of abnormal tetrads. Single tetrads with four equal-sized cells are rarely observed. The size and number of cells in a tetrad are indicative of the irregularity of distribution of chromosomes in both reduction divisions.

The chromosome behavior in the tetraploid hybrid *Triticum vulgare* \times *Secale montanum* differs very little from the triploid hybrid described above. Figure 3, F, shows an early anaphase with 28 univalent chromosomes. In G a bivalent may be seen among the scattered univalents, a condition not usually found.

A third hybrid, *Triticum vulgare* \times *Secale cereale*, was studied. Figure 3, H, shows a heterotypic prophase with the 28 chromosomes scattered through the spindle. The chromosome behavior during pollen formation does not seem to differ from that described in the two preceding hybrids except that no bivalent chromosomes were observed in the early heterotypic phases.

The tetraploid hybrid *Triticum spelta* \times *Secale montanum* showed as high as three bivalent chromosomes on the heterotypic metaphase plate. With the exception of this slightly greater pairing of the chromosomes, the behavior during the reduction divisions was as abnormal as that found in other *Triticum* \times *Secale* hybrids.

In Table 4 are listed the various investigators of the chromosomes in *Triticum* \times *Secale* hybrids. Two of these investigators, namely, Kihara (19) and Thompson (46), picture very clearly the more critical phases in the reduction divisions in pollen formation. Both investigators found from 0 to 3 bivalent chromosomes in the early phases of the heterotypic division. Table 4 shows that there is no appreciable amount of pairing in any of the *Triticum* \times *Secale* hybrids studied by the writers and other investigators. Thompson (46) found very little tendency for chromosomes to form a true metaphase plate. Kihara (19) observed a considerable number of chromosomes going at random to the poles, but also saw collected on the equatorial plate a group of univalents which split, the halves moving to the poles. The writers found many cases similar to those described by Kihara in which a somewhat definite equatorial plate was formed. All of the investigators seem to agree that the first division of *Triticum* \times *Secale* hybrids frequently produces more than two nuclei, which is a very different condition from that observed in *Triticum* \times *Triticum* hybrids.

In spite of the irregular behavior in the first division of these bigeneric hybrids, there is a tendency for the second division to function normally. Nuclei containing chromosomes unsplit in the first division divide regularly. The unsplit chromosomes in nuclei containing both split and unsplit chromosomes divide regularly, while the unsplit chromosomes pass at random to the poles or are extruded into the cytoplasm and form micronuclei. The writers' observations show that mother cells that produce more than two nuclei in the first division generally produce tetrads with more than

four cells, whereas if only two nuclei are formed in the first division, normal tetrads result. These cells, however, may have one or more nuclei, depending upon the character of the second division.

TABLE 4.—Summary of cytological work on *F*₁ *Triticum* × *Secale* hybrids

Hybrid combination		Chromosome number of parents		Chromosome pairing	Authority	Year
Female	Male	Female	Male			
<i>T. vulgare</i>	<i>S. cereale</i>	8	8		Nakao (24).....	1911
Do.....	do.....	21	7	0-3	Thompson (48).....	1926
Do.....	do.....	21	7	0-3	Kihara (19).....	1924
Do.....	do.....	21	7		Nikolaewa (28).....	1924
Do.....	do.....				Zalensky and Doroshenko (50)*	1924-25
Do.....	do.....	21	7	0	Present writers.....	
Do.....	<i>S. montanum</i>	21	7	0-1	do.....	
<i>T. spelta</i>	do.....	21	7	0-3	do.....	
<i>T. dicoccoides</i>	do.....	14	7	0	do.....	

* No counts or varieties indicated in English summary.

Plate 3 shows in a general way a series of characteristic meiotic phases. These photomicrographs do not attempt to illustrate the innumerable irregularities observed in the developing pollen mother cells, but represent a few of the more typical conditions arranged in a progressive order from the spireme to the early homotypic phases.

A spireme of *Triticum vulgare* × *Secale cereale* and a synoptic phase of *T. spelta* × *S. montanum* are shown in Plate 3. A and B were given very little detailed attention. The writers' studies were confined to the later phases in which the chromosomes were definitely formed and their number and behavior were clearly apparent. C and D show cells of *T. spelta* × *S. montanum* in the heterotypic prophase without any paired chromosomes. In C the chromosomes are scattered on the spindle; in D they are clumped together in the central region. E is a heterotypic anaphase of *T. spelta* × *S. montanum* showing the chromosomes, with one exception, collected in two groups at the poles, without any evidence of a metaphase plate. F shows a cell of *T. spelta* × *S. montanum* with two bivalents and a large number of univalents in the equatorial region and a few univalents at each pole. G shows a late heterotypic anaphase of *T. spelta* × *S. montanum* with a few chromosomes which failed to be included in the two major groups at the poles that have moved to the upper side of the spindle. H, of *T. spelta* × *S. montanum*, shows a cell in the same phase, but in this case a metaphase plate has been formed and the splitting univalents are assembled on the spindle. I and J are heterotypic telophases of *T. spelta* × *S. montanum*. The former shows three almost equal-sized chromatin masses, while the latter shows the formation of three nuclei, two of which are in the early telophase, the other nucleus being advanced to the late telophase. K shows a cell of *T. spelta* × *S. montanum* in which a large mass of undivided chromosomes have collected in the equatorial region and are being cut in two by the division of the cell. L, of *T. spelta* × *S. montanum*, shows a later phase of the phenomenon pictured in K. M and N, of *T. spelta* × *S. montanum*, and O, of *T. dicoccoides* × *S. montanum*, show typical cells produced by the abnormal heterotypic divisions.

CHROMOSOMES IN AEGILOPS SPECIES

A study of the chromosome number in the pollen mother cells of eight species of the genus *Aegilops* confirms the results of many of the investigators listed in Table 5. These species may be separated into diploid, tetraploid, and hexaploid groups, with seven as the reduced chromosome number in diploid species and multiples of this basic number in polyploid forms.

Aegilops squarrosa, as shown in Figure 7, A, has seven chromosomes at diakinesis. This number agrees with the determinations of Percival (32) and Sorokina (42). Kihara (19), Emme (8), Aase and Powers (1), and Kagawa (16) have found 14 haploid chromosomes in this species. The species examined by the latter investigators may have been *A. ventricosa*. The *A. ventricosa* examined by the writers was received originally under the name of *A. squarrosa*. These two species are morphologically different and may be easily separated.

There seems to be no dispute concerning the chromosome number for *Aegilops speltoides*. Figure 7, B, shows seven chromosomes on the heterotypic metaphase plate, and C shows a late anaphase with seven chromosomes nearing each pole.

Percival (31) is the only investigator that has not found 14 as the reduced chromosome number for *Aegilops cylindrica*. Figure 7, D, shows 14 chromosomes of *A. cylindrica* at diakinesis.

An early heterotypic anaphase in a pollen mother cell of *Aegilops ventricosa* is shown in Figure 7, E. Three chromosomes are still undivided, and 11 are seen going to each pole. Six other investigators (Table 5) have found this species to be tetraploid.

Aegilops ovata seems to be represented by a diploid and a tetraploid form, according to the recent studies of Schiemann (41). Other investigators (Table 5) picture 14 as the reduced chromosome number for this species. Figure 7, F, shows 14 chromosomes on each homotypic metaphase plate of *A. ovata*.

There seems to be no disagreement in assigning *Aegilops triuncialis* to the tetraploid group. Figure 7, G, shows a late heterotypic anaphase with 14 chromosomes at each pole. The plants for this study were grown from seed received as *A. triticoides*, but a careful study of the plants showed them to be *A. triuncialis*.

Aegilops triaristata is also a tetraploid species. Figure 7, H, shows the 14 chromosomes on the heterotypic metaphase plate. The same chromosome number for this species has recently been reported by Sorokina (42).

The chromosome number of *Aegilops crassa* seems to differ in different forms. Percival (32) found one form that is tetraploid and another that is hexaploid. Figure 7, I, shows a late heterotypic anaphase with 21 chromosomes at each pole. This number places the form studied by the writers in the hexaploid group. Emme (8), and more recently Sorokina (42), found 21 as the chromosome number in their material of this species.

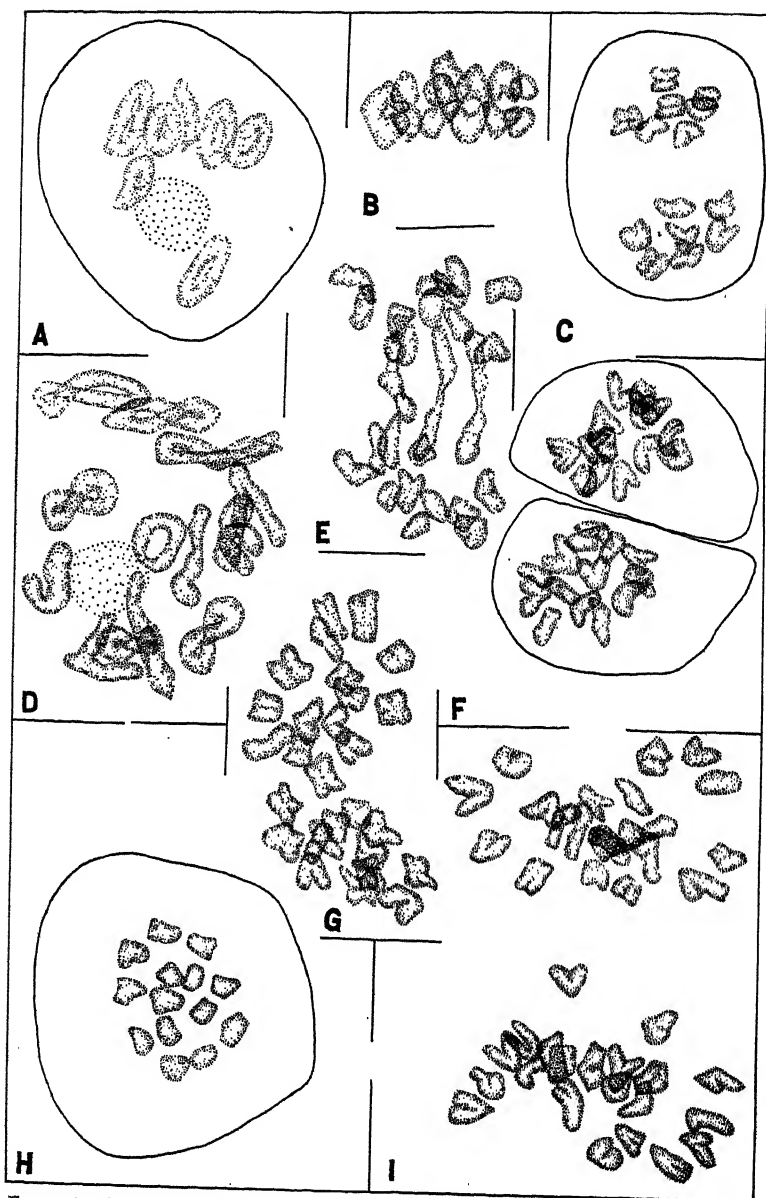


FIGURE 7.—Chromosomes in *Aegilops* species. $\times 2,000$: A, Diakinesis in *A. squarrosa* L.; B, heterotypic metaphase in *A. speltoides* Tausch.; C, heterotypic anaphase in *A. speltoides*; D, diakinesis in *A. cylindrica* Host.; E, early heterotypic anaphase in *A. ventricosa* Tausch.; F, homotypic metaphase in *A. ovata* L.; G, heterotypic anaphase in *A. triuncialis* L.; H, heterotypic metaphase in *A. triaristata* Willd.; I, heterotypic anaphase in *A. crassa* Boiss.

TABLE 5.—*Chromosome numbers of some Aegilops species as determined by the investigators named*
 [Diploid chromosome numbers in figure columns are shown in italics; all other numbers are haploid chromosomes]

Investigator	<i>A. ovata</i>	<i>A. trita- aristata</i>	<i>A. trita- uncialis</i>	<i>A. trita- coides</i>	<i>A. trita- uncialis</i>	<i>A. cy- lindrica</i>	<i>A. squar- rosa</i>	<i>A. cau- data</i>	<i>A. co- mosa</i>	<i>A. uni- aristata</i>	<i>A. ven- trifida</i>	<i>A. spel- toidea</i>	<i>A. ap- cheri</i>	<i>A. bi- cornis</i>	<i>A. crassa</i>	<i>A. lon- gissima</i>	<i>A. tur- comana</i>	<i>A. vari- abilis</i>
Bely, 1912 (5) 1919 (4) 1926 (30)	16	16																
Percival, 1923 (31), 1926 (32)	14	14	14	a 28		7	e 28				14	7			b 14			
Kihara, 1924 (49)	28, 14					14	e 28				28							
Sax and Sax, 1924 (40)	14					28	e 28				28							
Ernie, 1924 (8)	14		25	14		14	e 14											
Asse and Powers, 1926 (1)						14												
Gaines and Asse, 1926 (11)						14												
Tschernak and Bleier, 1926 (47)	14																	
Bleier, 1928 (7)	14	14	14		14	14	7	7			14	7	7	7	21	7	21	14
Sorokina, 1928 (42)	14	14	14		14	28	7	7			14	7	7	7	21	7	21	14
Schlemm, 1928 (41)	28, 14	14	14		28	28	e 14				14	7	7	7	21	7	21	14
Kagawa, 1928 (16)	14	14	14		28, 14	28, 14	7				14	7	7	7	21	7	21	14
Present writers																		

^a *A. triticoidea* is not considered a true species; the form examined by Kihara was probably *A. triticoidea*.

^b Another form of this species was examined with 21 haploid chromosomes.

^c It is probable that these investigators have confused *A. ventricosa* with *A. squarrosa*.

^d Another form of this species was examined with 7 haploid and 14 diploid chromosomes.

CHROMOSOMES IN F_1 HYBRIDS OF AEGILOPS SPECIES

The writers had cytological material available of one F_1 hybrid of *Aegilops* species. Heterotypic metaphases of pollen mother cells of *A. triuncialis* \times *A. ovata* show considerable pairing of chromosomes. The number frequently seen was 7, but lower numbers were common. After the bivalents divide, many of the univalents move to the plate and split longitudinally. (Pl. 1, G.) Heterotypic telophases show that all the chromosomes are included in the two daughter nuclei. The second reduction division shows many of the univalents that split in the first division moving tardily to the metaphase plate. (Pl. 1, H.) These univalents lag on the anaphase spindle, as shown in Plate 1, I. The lagging chromosomes pass at random to the two poles or remain behind and are left outside the major nuclei. There usually are only four pollen grains from a pollen mother cell. Some cells of the tetrad, however, may contain, in addition to the major nucleus, one or more minor nuclei. Only a limited number of *Aegilops* \times *Aegilops* hybrids have been studied, as shown in Table 6.

TABLE 6.—Summary of cytological work on F_1 *Aegilops* \times *Aegilops* hybrids

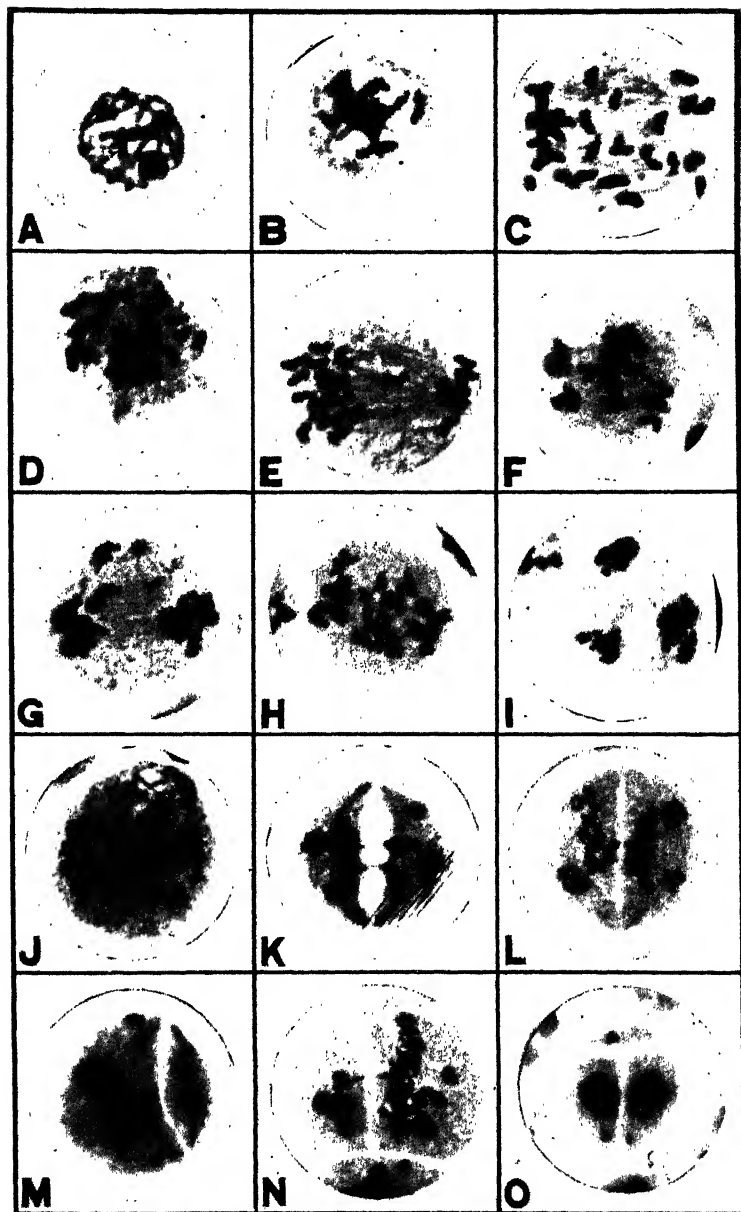
Hybrid combination		Chromosome number		Chromosome pairing	Authority	Year
Female	Male	Female	Male			
<i>A. ovata</i>	<i>A. caudata</i>	14	7	7-10	Bleier (?).....	1928
Do.....	<i>A. triuncialis</i>	14	14	0-7	Present writers.....	

Bleier (?) described a very similar condition for the triploid hybrid *Aegilops ovata* \times *A. caudata*. Heterotypic prophase show from 7 to 10 bivalent chromosomes and a few univalents. The latter split in the first division but were included in the two nuclei formed. Tetrads observed by him usually were 4-celled.

CHROMOSOMES IN HYBRIDS OF AEGILOPS \times TRITICUM

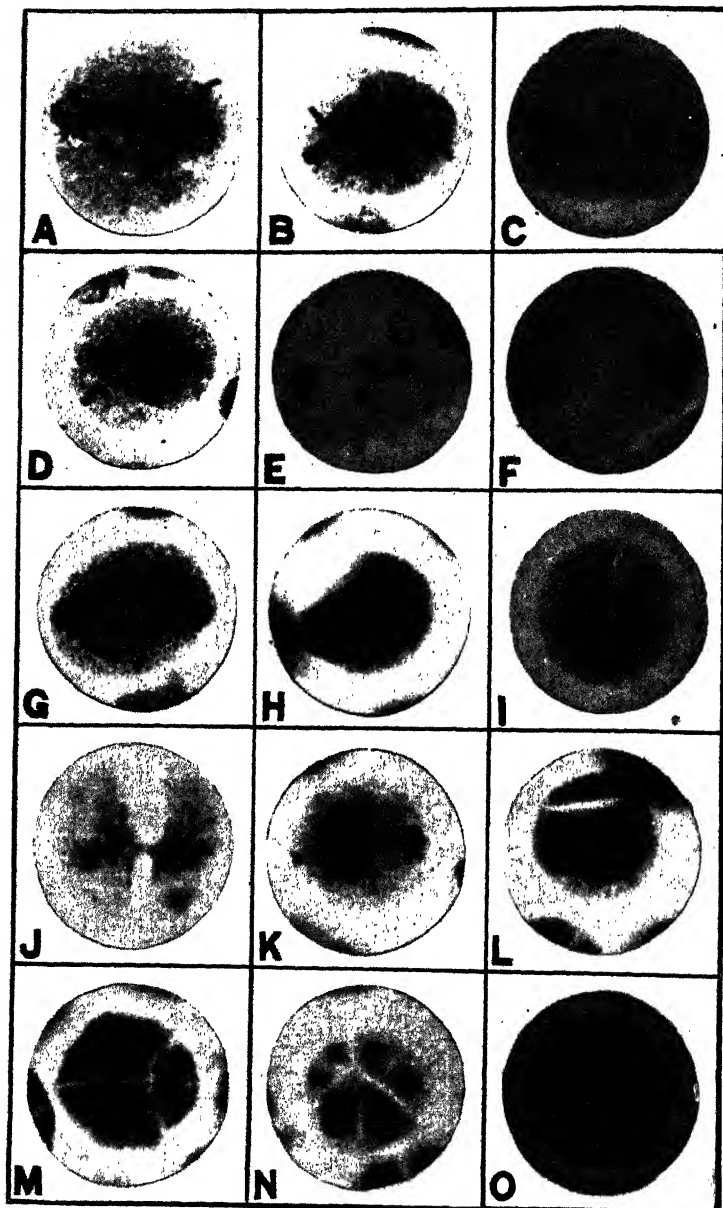
The writers had available cytological material for a study of the meiotic phases of the developing pollen mother cells of two tetraploid, five pentaploid, and three hexaploid *Aegilops* \times *Triticum* hybrids. Several F_1 plants of the cross *A. crassa* \times *T. monococcum* were produced but perished while quite young, thereby failing to provide material for cytological analysis.

The cross *Aegilops cylindrica* \times *Triticum turgidum* furnished a good series of the various meiotic phases. Figure 4, F and G; shows two characteristic prophase. F shows two bivalent and 24 univalent chromosomes, and G shows only univalent chromosomes. Three paired chromosomes were the largest number observed, and in a large percentage of the cells all the chromosomes were unpaired. Ordinarily many chromosomes collect at the metaphase plate and a few remain at either pole. The normal procedure is for chromosomes at the equatorial plate region to divide longitudinally and the halves move to the poles. In some cases, however, the central mass of chromosomes assumes a condition typical of the telophase stage. (Pl. 4, H.) A circle of chromatin material is formed at the plate region, which is divided into two parts by the formation of the cell walls. Occasionally,



PHOTOMICROGRAPHS OF MEIOTIC PHASES OF DEVELOPING POLLEN MOTHER CELLS IN TRITICUM X SECALE F_1 HYBRIDS. $\times 875$

A, Spireme in *T. vulgare* \times *S. cereale*; B, synapsis in *T. spelta* \times *S. montanum*; C, D, F, heterotypic prophase I in *T. spelta* \times *S. montanum*; E, G, H, heterotypic anaphase I in *T. spelta* \times *S. montanum*; I, J, heterotypic telophase I in *T. spelta* \times *S. montanum*; K, L, N, interkinesis in *T. spelta* \times *S. montanum*; M, N, early homotypic metaphase I in *T. spelta* \times *S. montanum*; O, interkinesis in *T. dicoccoides* \times *S. montanum*.



PHOTOMICROGRAPHS OF DEVELOPING POLLEN MOTHER CELLS IN F_1
AEGILOPS \times TRITICUM HYBRIDS. $\times 875$

A, B, D, Heterotypic metaphases in *A. crassa* \times *T. spelta*; C, E, F, heterotypic anaphases in *A. crassa* \times *T. dicoccum*; G, heterotypic telophase in *A. crassa* \times *T. spelta*; H, K, heterotypic metaphases in *A. cylindrica* \times *T. turgidum*; I, cell division in *A. crassa* \times *T. durum*; J, cell division in *A. crassa* \times *T. turgidum*; L, M, homotypic telophases in *A. cylindrica* \times *T. turgidum*; N, tetrad (?) in *A. crassa* \times *T. polonicum*; O, tetrad (?) in *A. crassa* \times *T. dicoccum*

however, at the time of the division of a cell, the dividing wall, instead of passing directly through the center and forming two equal-sized daughter cells, may circumflex the mass, leaving all of the chromatin material in one cell, while the other cell will be devoid of this substance. At the same time other cell walls may appear and cut off the nuclei formed by the chromosomes at the poles if some have remained there instead of joining the central mass. The cells produced in the heterotypic division in most cases divide regularly in the homotypic division, and most of the figures show no lagging of chromosomes on the spindle. This phenomenon suggests that univalent chromosomes rarely split and that the chromatin mass divides amitotically in the first division. The number of cells of the pollen tetrads vary, depending on the character of the heterotypic division.

The second tetraploid hybrid, *Aegilops cylindrica* \times *Triticum polonicum*, shows during meiosis no noticeable differences from that described for the previous hybrid. Figure 4, H, shows the chromosomes from a pollen mother cell with three paired chromosomes, while I shows only univalents scattered through the spindle.

In the pentaploid hybrid *Aegilops crassa* \times *Triticum turgidum* the chromosomes are scattered through the spindle region in the prophase. Frequently these chromosomes all appear as univalents, but sometimes as high as four bivalents were observed, as shown in Figure 4, B. This amount of pairing is slightly more than that noted in any of the tetraploid hybrids. The chromosome behavior in the later stages is similar in every detail to that described for the two previous hybrids. The tetrads resulting from such meiotic behavior are irregular, and the cells vary both in number and size.

The pentaploid hybrid *Aegilops crassa* \times *Triticum polonicum* shows no outstanding differences in chromosome behavior from the preceding pentaploid hybrid. Figure 8, C, is a prophase stage showing 35 univalent chromosomes, and J is a similar phase showing 3 bivalent chromosomes. D is a later phase showing univalent chromosomes at the two poles and a large group at the plate. All of the latter chromosomes show some indications of undergoing a longitudinal division. A few cases were noticed in which those at the equatorial plate have split and moved to join the groups at the poles. The more usual condition is shown in E, with one chromosome remaining at the pole, while the others have moved to the equatorial region, where they have taken on the appearance of chromosomes in a telophase stage. In the process of cell division as shown in E there may be seen at one end a cell containing one chromosome and a second wall in the equatorial region in the process of dividing the chromatin mass into two large cells with equal quantities of chromatin material. In F and G are shown cells resulting from irregular heterotypic divisions. F shows one cell with a single split chromosome and two cells with slightly unequal amounts of chromatin material. H shows a 6-celled tetrad resulting from such abnormal chromosome behavior as shown in F.

Hybrids of *Aegilops crassa* \times *Triticum durum* show in typical prophases (fig. 9, H) 35 unpaired chromosomes, while only a few cases with bivalent chromosomes were seen. In Figure 9, D-G, are shown the usual method of chromosome and cell division, a true heterotypic division being rarely observed.

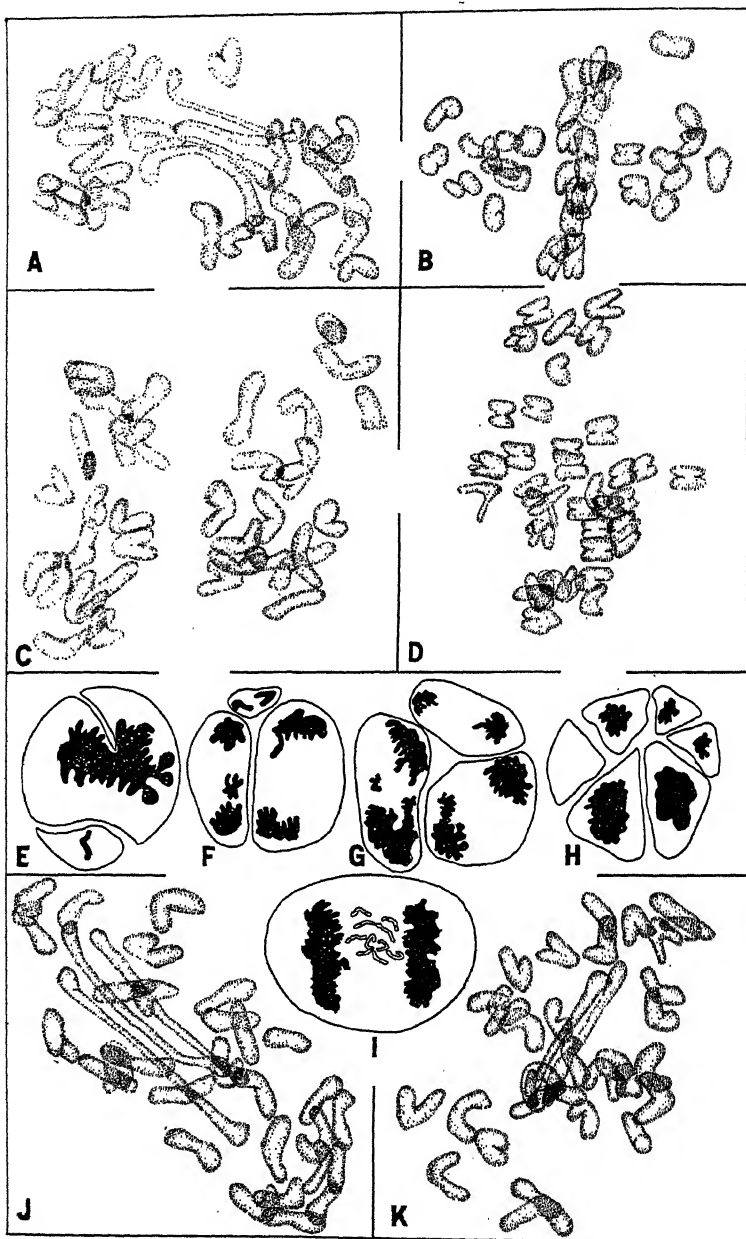


FIGURE 3.—Chromosomes in *Aegilops* \times *Triticum* hybrids: A, Heterotypic metaphase in *A. crassa* \times *T. dicoccoides*; B, heterotypic anaphase in *A. crassa* \times *T. dicoccoides*; C, heterotypic prophase in *A. crassa* \times *T. polonicum*; D, heterotypic anaphase in *A. crassa* \times *T. polonicum*; E, interkinesis in *A. crassa* \times *T. polonicum*; F, late homotypic anaphase in *A. crassa* \times *T. polonicum*; G, late homotypic anaphase in *A. crassa* \times *T. polonicum*; H, tetrad in *A. crassa* \times *T. polonicum*; I, heterotypic anaphase of F_2 of *A. ovata* \times *T. dicoccum*; J, heterotypic metaphase in *A. crassa* \times *T. polonicum*; K, heterotypic metaphase in *A. crassa* \times *T. dicoccum*. A–D, J, $\times 2000$; E–I, $\times 875$.

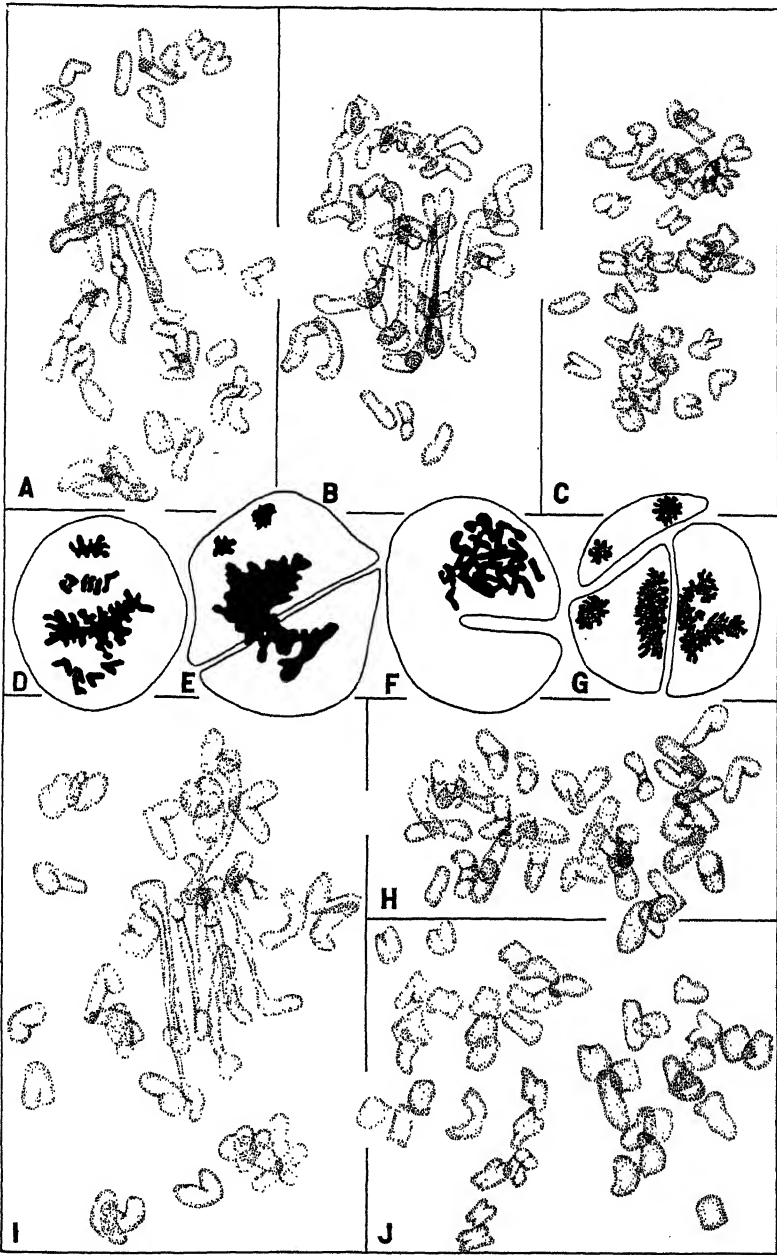


FIGURE 9.—Chromosomes in *Aegilops* \times *Triticum* hybrids: A, Heterotypic metaphase in *A. crassa* \times *T. spelta*; B, heterotypic metaphase in *A. crassa* \times *T. vulgare*; C, heterotypic anaphase in *A. crassa* \times *T. vulgare*; D, heterotypic anaphase in *A. crassa* \times *T. durum*; E, F, G, interkinesis in *A. crassa* \times *T. durum*; H, heterotypic prophase in *A. crassa* \times *T. durum*; I, heterotypic metaphase in *A. crassa* \times *T. compactum*; J, heterotypic anaphase in *A. crassa* \times *T. compactum*. A-F, H-J, $\times 2000$; D-G, $\times 875$

Figure 9, D, shows the distribution of the chromosomes into four groups, while E is a later phase showing the cell wall cutting through the major centrally located chromosome mass. In F, which is unusual, the cell wall, instead of passing through the chromosomes, passes to one side, forming one cell without a nucleus. In G a later phase is shown. It is difficult to say definitely what phase it represents, but it appears to be a late heterotypic phase, derived from a cell which passed through a stage similar to that shown in E. If so, one expects each chromosome mass to split equally in the homotypic division and produce either 6 pollen grains, 4 of which will be binucleated; 8 pollen grains, 2 of which will be binucleated; or 10 mononucleated pollen grains.

The fourth pentaploid hybrid, *Aegilops crassa* \times *Triticum dicoccum*, appears in most reduction phases to be identical with the preceding hybrids. There seems, however, to be a somewhat greater tendency for the chromosomes to pair. Figure 4, A, and Figure 8, K, show heterotypic metaphases with two bivalent chromosomes. Some cells were observed with as many as six pairs of chromosomes.

The description of the meiotic phases in the pentaploid hybrid *Aegilops crassa* \times *Triticum dicoccoides* was purposely reserved for consideration until the last. In it was found a chromosome behavior quite distinct from that described for the preceding hybrids and similar to that described for *Triticum* \times *Triticum* hybrids.

The number of paired chromosomes usually seen was four. Figure 8, A, shows a typical distribution of the chromosomes at the early metaphase. B is a typical picture of a later phase, showing the divided halves of bivalents with a few univalents at each pole and a large number of univalents assembled on the equatorial plate preparing to split longitudinally, after which operation they separate and join the two groups at the poles. At interkinesis, therefore, there are produced two equal-sized mononucleated cells, each nucleus containing two types of chromosomes. The homotypic division is irregular, due to the presence of the split univalents. The homotypic anaphase shows many of them on the spindle, going at random to the poles, and frequently a few, too late to be included in the daughter nuclei, are left in the cytoplasm to form micronuclei.

Pollen tetrads, with rare exceptions, are four celled. Each cell, however, may have micronuclei in addition to the major nucleus. A count of 43 cells showed 35 with one nucleus only, 6 with a major and a minor nucleus, and 1 with a major and two minor nuclei.

It is very apparent that the behavior of the chromosomes in this *Aegilops* \times *Triticum* hybrid is similar in every respect to that earlier described for *Triticum* \times *Triticum* hybrids. Such a chromosome behavior during the meiotic phases suggests that *T. dicoccoides*, the so-called "wild wheat" of Palestine, shows somewhat the same cytological relation to *A. crassa* as *T. monococcum* shows in relation to other species of *Triticum*.

The hexaploid hybrid *Aegilops crassa* \times *Triticum compactum* shows in the early heterotypic phases as high as 7 paired chromosomes. Figure 9, I, shows a cell with 7 dividing bivalent chromosomes and 28 univalents. J shows a later phase with two groups approaching the poles and a few univalents on the equatorial plate. There seems to be a greater tendency in this hybrid than in the earlier described *Aegilops* \times *Triticum* hybrids for a normal division of the chromo-

somes into two equal masses and the production of two cells in the heterotypic division. Irregularities that produce three or even more cells are abundant, however, and the general chromosome behavior is that described as typical for hybrids between these two genera. The chromosomes in the two to several nuclei divide equally in the homotypic division, giving tetrads with varying numbers of pollen cells.

In the hybrid *Aegilops crassa* \times *Triticum vulgare* the condition is very similar to that described in the previous hexaploid hybrid. Four to seven bivalents are most frequent. Figure 9, B, shows a typical early metaphase, while C shows a later phase very similar to that in J of the *A. crassa* \times *T. compactum* cross.

Figure 9, A, shows a typical cell of the hybrid *A. crassa* \times *T. spelta* with 4 bivalents and 34 univalents.

Hexaploid hybrids in general show more chromosomes pairing and a more normal distribution of chromosomes than are found in tetraploid or pentaploid *Triticum* \times *Aegilops* hybrids. Tetrad formation, however, is more irregular than that found in the exceptional hybrid *A. crassa* \times *T. dicoccoides*. The chromosome behavior in hybrids of *Aegilops* \times *Triticum* is similar, but is strikingly different from that found in *Aegilops* \times *Aegilops* and *Triticum* \times *Triticum* hybrids.

Plate 4 has been prepared to show by means of a series of photomicrographs the developing pollen mother cells of *Aegilops* \times *Triticum* hybrids.

Plate 4, A and B, are heterotypic metaphases of *Aegilops crassa* \times *Triticum spelta* in which some of the chromosomes are in the equatorial region and others are scattered through the spindle. In B two bivalents are shown among the univalents in the group at the plate. C shows a heterotypic anaphase of *A. crassa* \times *T. dicoccum* with two groups of chromosomes at each pole and a dividing bivalent stretched between them. D shows a cell of *A. crassa* \times *T. spelta* with two groups of chromosomes at the poles and a third group of univalents on the equatorial plate. E shows a later phase of a cell of *A. crassa* \times *T. dicoccum* in which the split univalents are moving toward the chromosomes at the poles, and F shows these split univalents very close to the two groups of chromosomes that preceded them to the poles. G shows a telophase of *A. crassa* \times *T. spelta* in which the chromosomes, with the exception of two showing faintly on the lower edge of the spindle, are included in two major chromatin masses. H-K show a more abnormal heterotypic division phenomenon. In H all the chromosomes in a cell of *A. cylindrica* \times *T. turgidum* are shown in a central position, but instead of undergoing division they have reverted to a condition that is characteristic of chromosomes in the early telophase. K is a cell of *A. cylindrica* \times *T. turgidum* in the same phase, but shows in addition to the chromatin mass at the center a few chromosomes at each pole. I shows in *A. crassa* \times *T. durum* a centrally located chromatin mass being cut in two by the division of the cell in the formation of a dyad. J shows a later phase of *A. crassa* \times *T. turgidum* with a chromatin strand still bridging the gap between the two cells. L and M show homotypic anaphases of typical pollen mother cells of *A. cylindrica* \times *T. turgidum*. In the former a regular division of the chromosomes of the two unequal-sized nuclei takes place, while in the latter a regular division of

the three nuclei occur. N, of *A. crassa* × *T. polonicum*, and O, of *A. crassa* × *T. dicoccum*, show pollen tetrads with 6 and 7 cells, respectively.

Table 7 lists the investigators who have made cytological studies of the chromosome behavior in developing pollen mother cells of *Aegilops* × *Triticum* hybrids.

TABLE 7.—Summary of cytological work on *F*₁ *Aegilops* × *Triticum* hybrids

Hybrid combination		Chromosome number		Chromosome pairing	Authority	Year
Female	Male	Female	Male			
<i>A. ovata</i>	<i>T. monococcum</i>	14	7	0-5	Bleier (?).....	1928
Do.....	<i>T. dicoccum</i>	14	14	0-7	Percival (32).....	1926
Do.....	do.....	14	14	0	Sax (39).....	1928
Do.....	<i>T. vulgare</i>	14	21	0-3	Bleier (?).....	1928
Do.....	do.....	14	21	0-few	Percival (32).....	1926
<i>A. cylindrica</i>	<i>T. durum</i>	14	14	0	Bleier (?).....	1928
Do.....	<i>T. turgidum</i>	14	14	0	Gaines and Aase (11).....	1926
Do.....	do.....	14	14	0-3	Present writers.....
Do.....	<i>T. polonicum</i>	14	14	0-3	do.....
Do.....	<i>T. vulgare</i>	14	21	5-7	Sax and Sax (40).....	1924
Do.....	do.....	14	21	7	Kagawa (16).....	1928
Do.....	<i>T. spelta</i>	14	21	7	Bleier (?).....	1928
<i>A. crassa</i>	<i>T. dicoccoides</i>	21	14	0-5	Present writers.....
Do.....	<i>T. dicoccum</i>	21	14	0-6	do.....
Do.....	<i>T. durum</i>	21	14	0-3	do.....
Do.....	<i>T. turgidum</i>	21	14	0-4	do.....
Do.....	<i>T. polonicum</i>	21	14	0-4	do.....
Do.....	<i>T. compactum</i>	21	21	0-7	do.....
Do.....	<i>T. vulgare</i>	21	21	0-7	do.....
Do.....	<i>T. spelta</i>	21	21	0-6	do.....
<i>T. durum</i>	<i>A. ovata</i>	14	14	Kagawa (16).....	1928
<i>T. vulgare</i>	do.....	8	16	Bally (4).....	1919
Do.....	do.....	21	14	Emme (8).....	1924
Do.....	<i>A. cylindrica</i>	21	14	7	Gaines and Aase (11).....	1926
Do.....	do.....	21	14	7	Kagawa (16).....	1928

Bally (4) studied the meiotic phases in the pentaploid hybrid *Triticum vulgare* × *Aegilops ovata*. His study was the first of its kind, and though his chromosome counts were not accurate, as has since been proved by other investigators, his observations on the chromosome behavior give a clear general picture of the irregular chromosome distribution in both the heterotypic and homotypic divisions. The irregular tetrads shown by him are typical of *Aegilops* × *Triticum* hybrids. The reciprocal cross (*A. ovata* × *T. vulgare*) has been studied more recently by Percival (32) and Bleier (?). The number of paired chromosomes has been found by them to be small, and the tetrad formation generally irregular.

Bleier (?) was the first to study triploid *Aegilops* × *Triticum* hybrids. In the *F*₁ of an *A. ovata* × *T. monococcum* hybrid 21 univalents usually are seen, but a few cases show as many as 5 bivalent chromosomes.

Both Percival (32) and Sax (39) have studied the chromosome behavior in *Aegilops ovata* × *T. dicoccum*. Percival finds little, if any, pairing of chromosomes in the heterotypic prophase; Sax finds no pairing. Both, however, find tetrad formation very irregular, giving microspores of various sizes and in varying numbers. Gaines and Aase (11) observed no chromosome pairing in the *F*₁ hybrid of *A. cylindrica* × *T. turgidum*. Bleier (?) has described some of the meiotic phases in the tetraploid hybrids of *A. cylindrica* × *T. durum*.

No pairing was observed, and all univalent chromosomes go at random to the poles without splitting.

Sax and Sax (40), Gaines and Aase (11), and more recently Kagawa (16) have studied the meiotic phases in pollen mother cells of F_1 hybrids between *Triticum vulgare* and *Aegilops cylindrica*. All of the investigators found 7 bivalent and 21 univalent chromosomes in the heterotypic phases. Kagawa found that the univalents sometimes split in the heterotypic division, these monads being distributed at random to the poles in the homotypic division or left in the cytoplasm. In another pentaploid hybrid, *A. cylindrica* \times *T. spelta*, Bleier (?) found 7 paired chromosomes in the heterotypic prophase and no indication of univalents splitting in the first division.

Table 7 has been prepared to show the amount of chromosome pairing observed by the writers and other investigators in the early heterotypic phases of *Aegilops* \times *Triticum* hybrids. It is apparent that seven paired chromosomes is the maximum number found. In most cases, however, the number of paired chromosomes is variable and seldom is as high as seven.

In conjunction with the cytological investigations with F_1 hybrids herein discussed, four F_3 self-pollinated plants of the cross *Aegilops ovata* \times *Triticum dicoccum* were studied. In all of these F_3 plants the chromosome number exceeded that of the F_1 , but in none was there any indication of a true doubling like that found by Tschermak and Bleier (47) in F_5 and F_8 hybrids of *A. ovata* \times *T. dicoccoides* and *A. ovata* \times *T. durum*.

In the F_3 material studied by the writers, two plants from the same F_1 plant line had 26 and 27 haploid chromosomes, respectively. Another plant from a different F_1 plant line had 25 haploid chromosomes in the pollen mother cells. One other plant was studied in which no chromosome counts were established but in the homotypic division of which from 5 to 6 lagging chromosomes were observed. In the 26-chromosome plant one cell had 26 pairs only, one cell had 25 pairs and 2 splitting univalents, another cell had 22 pairs and 8 splitting univalents, while still another cell had 19 pairs and 14 splitting univalents. In the 25-chromosome plant one cell had 25 pairs only, and one cell had 22 pairs, 3 splitting univalents, and 3 unsplit univalents toward one pole. In each of two other cells 5 univalents were observed splitting, the pairs not being counted. In the 27-chromosome plant 3 cells were observed each with 26 pairs and 2 splitting univalents.

Hybrids between *Aegilops* and *Triticum* have been obtained and the successive generations continued for many years by several investigators. Practically all of the hybrids obtained, however, have been subjected to cross-pollination with wheat. Apparently no carefully controlled self-pollination experiments were conducted with F_1 hybrids of such crosses. In order to determine the extent of self-fertility in *Aegilops* \times *Triticum* hybrids several F_1 plants of the hybrid *A. ovata* \times *T. dicoccum* were inclosed in glassine bags during the pollination period by the junior writer. A small percentage of seeds was obtained under these conditions. During the subsequent generations the plants were again self-pollinated under controlled conditions. The cytological studies on the F_3 hybrids of the cross *A. ovata* \times *T. dicoccum* have been made from such controlled self-pollinated material.

In 1926 Tschermak and Bleier (47) made cytological studies of fertile F_5 F_6 plants of the hybrids *Aegilops ovata* \times *Triticum dicoccoides* and *A. ovata* \times *T. durum*. Twenty-eight haploid chromosomes were observed in the plants of these hybrids. This doubling was attended by a regular reduction division in the pollen mother cells unlike that found in hybrids. The somatic cells were observed to have 56 chromosomes. In addition, however, to the regular cytological phenomena Tschermak and Bleier (47) frequently observed small strongly stained bodies in the cytoplasm of the pollen tetrad cells which they indicated might be extruded chromosomes. When an F_5 F_6 hybrid of the first cross (*A. ovata* \times *T. dicoccoides*) was crossed with a hybrid of the second cross (*A. ovata* \times *T. durum*), the reduced chromosome number was 28 and the reduction divisions were quite normal. In further investigations Bleier (?) backcrossed *A. ovata* with the fertile F_5 F_6 hybrids of *A. ovata* \times *T. dicoccoides* and *A. ovata* \times *T. durum*. These new F_1 hybrids produced pollen mother cells with 14 bivalent and 14 univalent chromosomes, thus indicating that the male gametes used contained 28 chromosomes.

In the cytological studies by Sax (39) of six plants resulting from a cross of the F_1 *A. ovata* \times *T. dicoccum* hybrid with the *T. dicoccum* parents, 28 chromosomes were established as the complement of the egg cells in the F_1 .

Though a tendency toward doubling of chromosomes was observed by the writers in F_3 hybrid material of a cross between *Aegilops ovata* and *Triticum dicoccum*, the individual plants studied showed from 1 to 3 chromosomes less than a true double number 28. It was characteristic of these plants to form tetrads whose individual cells contain one or more micronuclei besides a major nucleus. Phenomena of this kind appear generally to be the result of irregular behavior of chromosomes in the meiotic phases preceding the formation of tetrads and suggest the presence of nonhomologous chromosomes. It seems quite probable that what appeared to Tschermak and Bleier as strongly stained bodies in the cytoplasm of the cells of the tetrads in their hybrids may have been similar to the micronuclei or extruded chromosomes observed by the writers in their material.

Figure 4, C, shows 26 chromosomes at each pole in the heterotypic metaphase of one of the F_3 plants referred to above. The chromosome behavior in all of the above F_3 hybrids studied was more or less irregular, but was more regular than in any of the F_1 *Aegilops* \times *Triticum* hybrids previously described. The phenomena generally observed indicate that a large number of chromosomes paired and formed a fairly regular metaphase plate, but some unpaired chromosomes were usually present. Most of the univalents usually split in the heterotypic division (fig. 8, I), and the halves occasionally failed to be included in the major nuclei. The more common procedure, however, is for the univalents to split, the halves going to separate poles, where they are included in the nuclear mass composed of the members of paired chromosomes. The homotypic division often showed halves of univalents lagging on the spindle, some of which failed to be included in the major nuclei but formed one to several micronuclei.

Typical phases of the pollen mother-cell development are illustrated in six photomicrographs shown in Plate 1. A heterotypic anaphase is shown in J, in which practically all chromosomes have

paired and divided. K shows a heterotypic telophase with an extruded chromosome at the lower side of the spindle. L shows a typical homotypic anaphase with several chromosomes lagging on the spindle. Three quite different tetrads are shown in M-O. The first is a tetrad in which the nuclei of two cells are much larger than those of the other two. The second shows micronuclei in three of the cells of the tetrad. The third is a pentad, in which four cells are mononucleated and one cell has a micronucleus in addition to the major nucleus.

DISCUSSION

The chromosome number and general meiotic behavior of the various species of the genera *Triticum*, *Secale*, and *Aegilops* examined by the writers were similar with few exceptions, to those observed by other investigators. The lack of agreement in the chromosome counts of certain species is doubtless due to an improper identification of the species. All of the *Triticum* × *Triticum* hybrids studied had a diploid species (*T. monoccocum*) as the pollen parent, the female parent being either a tetraploid or a hexaploid species. In such hybrids, which combine species with different chromosome numbers, both paired and unpaired chromosomes were observed. In all of the crosses, however, the amount of chromosome pairing varied from 0 to 7 and showed no indication that a definite number of paired chromosomes was characteristic in any of the hybrids studied.

The unpaired chromosomes were erratic in their behavior, splitting either in the first or in the second reduction division. These split univalent chromosomes generally are included in the two daughter nuclei formed from the first division, but in their random movement to the poles in the second division they frequently lag behind on the spindle and fail to be included in the four major nuclei. These extruded chromosomes form micronuclei, and consequently many cells of the tetrads are polynucleated.

These results are not altogether corroborated by the work of earlier investigators. Thompson (45) found a chromosome situation in a triploid *Triticum* hybrid corresponding quite closely to that herein described. The chromosome behavior of pentaploid hybrids described by Kihara (17, 19) and Melburn and Thompson (22) is different from that found in triploid and tetraploid hybrids studied by the writers. Those investigators found chromosomes left in the cytoplasm at the conclusion of both the heterotypic and the homotypic divisions. The writers find this phenomenon very characteristic of the homotypic division but very rare in the heterotypic division. Sax (37) described a chromosome behavior of some F_1 *Triticum* × *Triticum* hybrids that differs quite noticeably from the studies of more recent investigators, but there seems to be quite general agreement in the character of the tetrads. *Triticum* × *Triticum* hybrids, with few exceptions, produce 4-celled tetrads; each cell, however, may have one or more minor nuclei in addition to the major nucleus. In hybrids between species of the emmer group and species of the *vulgare* group a constant number of 14 pairs of chromosomes has been observed by other investigators. Such variable pairing as is indicated in the tetraploid hybrids studied by the writers suggests a more distant relationship between the einkorn group and both the emmer and *vulgare* groups than that existing between the emmer and *vulgare* groups.

The chromosome behavior in *Aegilops* × *Triticum* hybrids, with one exception, is similar; i. e., individual differences are apparent but these are often insignificant. The amount of pairing of chromosomes varies from 0 to 7. This variability agrees with the results of recent investigators. Sax and Sax (40) and Gaines and Aase (11), however, find 7 paired and 14 unpaired chromosomes the general rule in the early heterotypic phases of an *A. cylindrica* × *T. vulgare* hybrid. Only a few cases have been reported with such a definite number of pairs. Crosses of *A. cylindrica* × *T. vulgare* and *A. cylindrica* × *T. spelta* seem to come the nearest to having 7 definitely paired chromosomes, but even in these two crosses lower numbers are reported. The writers' studies of *Aegilops* × *Triticum* hybrids show the number of paired chromosomes to be small and variable.

A chromosome behavior different from that which is characteristic of *Aegilops* × *Triticum* hybrids was found by the writers in the study of the meiotic phases of the hybrid *A. crassa* × *T. dicoccoides*. The chromosomes in this hybrid were distributed during the reduction division in a manner similar to that observed in *Triticum* × *Triticum* hybrids and unlike that of the other *Aegilops* × *Triticum* hybrids studied. This exceptional behavior suggests a closer relationship between *T. dicoccoides* and *A. crassa* than between other *Triticum* species and *A. crassa*. The extent of the pairing in hybrids between these two genera is similar to that observed in hybrids between einkorn and members of the two polyploid *Triticum* groups, but the lower amount of pairing suggests a more distant relationship between these genera than that between the *Triticum* groups described above. The more recent studies of chromosome pairing in hybrids between *Aegilops* and *Triticum* give little support to that portion of the hypothesis proposed by Gaines and Aase (11) and illustrated in Table 8 to explain the relationship of *Aegilops* to *Triticum*.

TABLE 8.—Chromosome sets contained in *Triticum* and *Aegilops*

Group or species	Gaines and Aase, sets of 7 chromosomes				Present writers, sets of 7 chromosomes				
	A	B	C	D	A	B	C	D	E
Einkorn	×				×				
Emmer	×	×			×	×			
Vulgare	×	×	×		×	×	×		
<i>Aegilops cylindrica</i>			×	×		×		×	
<i>A. ovata</i>					×			×	
<i>A. crassa</i>						×		×	×

The inconsistency of pairing of the A sets of chromosomes in hybrids between einkorn and members of the emmer and *vulgare* groups suggests that the chromosomes in the A sets of the above groups may be similar in some respects but not identical. On the other hand, the consistent pairing of the A and B sets of chromosomes in hybrids between members of the emmer and *vulgare* groups seems to indicate that the chromosomes are more nearly alike.

When *Aegilops ovata* was crossed with einkorn the same inconsistent pairing phenomenon was exhibited as that observed in hybrids between einkorn and members of the emmer and *vulgare* groups. The A set of chromosomes in *A. ovata* does not appear to be identical

with that in einkorn or members of the emmer and *vulgare* groups. Neither does it appear that the B set of chromosomes in the *vulgare* group is identical with that in the emmer group and in *A. cylindrica* and *A. crassa*.

Even more complex relationships exist in the *Aegilops* groups. From the recent cytological evidence apparently none of the sets in the *Aegilops* are exactly the same as those in the *Triticum* groups. The set in *A. cylindrica* now placed under B by the writers is the set previously placed under C by Gaines and Aase (11). This change is suggested by the pairing of chromosomes (Table 7) observed in hybrids between *A. cylindrica* and species of the emmer group. By consulting Tables 7 and 8 it may be seen that in hybrids between *Aegilops* and *Triticum* there is little if any suggestion of the presence of a C set of chromosomes. It is more apparent, however, from the pairing observed in *A. cylindrica* \times *T. turgidum* and *T. polonicum* hybrids that some chromosomes of both A and B sets are present.

From the complete and partial incompatibility of chromosomes exhibited in hybrids resulting from a combination of parents with different numbers of chromosomes, it appears that in any two species only a few chromosomes or probably none at all are identical.

If the *vulgare* wheats were derived by the combination of *Aegilops cylindrica* or *A. ovata* with species of the emmer group, as has been suggested as probable by Percival (30), such a condition as that described above makes it difficult to reconcile the cytological results with Percival's view.

The cytological studies of the writers with F_3 hybrid material of the cross *Aegilops ovata* \times *Triticum dicoccum* have given evidence of a lack of a true doubling of the chromosome number. If a true doubling takes place like that observed by Tschermak and Bleier (47) in their F_3 *Aegilops* \times *Triticum* hybrids, the individual plants should produce gametes with 28 chromosomes. None of the F_3 hybrid material of the cross *A. ovata* \times *T. dicoccum* examined by the writers has exhibited the doubling phenomenon observed by Bleier, but on the other hand it produced individuals the reduced chromosome number of which was 25 to 27.

A casual inspection of the morphological characters of plants of the F_1 , F_2 , and F_3 gives the impression that the F_1 type is perpetuated throughout the F_2 and F_3 generations and that no segregation takes place. Limited detailed observations on several plant characters, however, indicate that measurable significant variations may occur among individuals within the F_2 and F_3 generations. From the cytological study of several individuals of the F_3 herein described, a more marked variation in the chromosome behavior is exhibited than that expressed by the morphological character of the same plants. From this it appears that the absent chromosomes do not contain any factors that contribute toward the expression of the major morphological characters exhibited by these individuals. From the meager cytological observations made by the writers with F_3 plants containing from 25 to 27 chromosomes it appears that individuals having a haploid chromosome complement of 1 to 3 less than 28 may exhibit little variation in morphological characters.

That elimination of certain forms with different morphological characters and chromosome numbers takes place and may be expressed

in several ways is suggested by the following observations on self-pollinated F_1 , F_2 , and F_3 plants of the hybrid *Aegilops ovata* \times *Triticum dicoccum*:

- (1) Completely sterile, partly sterile, and practically fertile plants have been produced.
- (2) No plants have been examined with a chromosome number of less than 25.
- (3) A large percentage of apparently normal seeds fails to germinate.
- (4) Some plants are stunted or succumb before reaching maturity.
- (5) Microscopical examination of the contents of the anthers of various plants shows some plants with almost perfectly developed pollen, whereas others contain imperfectly developed pollen.

SUMMARY

The haploid chromosome number in species of *Secale*, *Triticum*, and *Aegilops* is seven or a multiple of this basic number.

The chromosome behavior during meiosis of the developing pollen mother cell in species of these three genera is regular, producing normal tetrads with each cell receiving the same number of chromosomes.

Very few chromosome abnormalities were observed in hybrids of *Secale cereale* \times *S. montanum*.

Pollen mother cells of triploid and tetraploid wheat hybrids show the following abnormalities in chromosome behavior:

Univalent and a few bivalent chromosomes are present in variable numbers in the early heterotypic phases.

Many of the univalents split longitudinally in the heterotypic division.

Only two nuclei and two cells are produced by the heterotypic division.

The homotypic division is characterized by a random distribution and frequent extrusion of the halves of univalent chromosomes that split in the previous division.

Extruded chromosomes frequently form micronuclei.

Tetrads are 4-celled, but many of the cells are polynucleated.

The chromosome behavior in *Triticum* \times *Secale* and *Aegilops* \times *Triticum* hybrids shows the following abnormalities:

Univalent and a few bivalent chromosomes are present in varying numbers in the early heterotypic phases.

Occasionally univalents split longitudinally in the heterotypic division.

Frequently univalents collect in the center of the cell without any indication of a mitotic division. This centrally located chromatin mass, however, is often divided in an amitotic manner by the forming cell wall.

The number and size of the cells produced by the heterotypic division is variable.

The second reduction division is more regular than the first. Whenever univalents are present in the chromosome complement they are distributed at random to the poles or are extruded into the cytoplasm.

Tetrads frequently have more than four cells, due to the irregular heterotypic division. Some of the cells of the tetrad may be polynucleated.

The hybrid *Aegilops crassa* \times *Triticum dicoccoides* has a chromosome behavior more like that described for *Triticum* \times *Triticum* hybrids than that of *Aegilops* \times *Triticum* hybrids.

This exceptional behavior in an *Aegilops* \times *Triticum* hybrid suggests that *T. dicoccoides* is more closely related than other *Triticum* species to the genus *Aegilops*.

F_3 plants of the cross *Aegilops ovata* \times *Triticum dicoccum* indicate a tendency toward doubling of chromosome numbers. The few plants examined had from 25 to 27 haploid chromosomes.

The variable chromosome pairing found in hybrids of *Triticum monococcum* and members of the emmer and *vulgare* groups suggests a distant relationship between this species and the polyploid *Triticum* groups. A more distant relationship than that mentioned above

appears to exist between species of the genus *Aegilops* and the genus *Triticum*, as is indicated by the chromosome behavior in hybrids between these genera.

The chromosome behavior in *Triticum* × *Secale* hybrids suggests an even more distant relationship between these two genera than between the genera *Triticum* and *Aegilops*.

LITERATURE CITED

- (1) AASE, H. C., and POWERS, LE R.
1926. CHROMOSOME NUMBERS IN CROP PLANTS. *Amer. Jour. Bot.* 13: 367-372, illus.
- (2) ASCHERSON, P. and GRAEBNER, P.
1892-1902. SYNOPSIS DER MITTELEUROPÄISCHEN FLORA. Bd. 2, Abt. 1. Leipzig.
- (3) BALLY, W.
1912. CHROMOSOMENZAHLEN BEI TRITICUM UND AEGILOPSARTEN. EIN CYTOLOGISCHER BEITRAG ZUM WEIZENPROBLEM. *Ber. Deut. Bot. Gesell.* 30: 163-172, illus.
- (4) ———
1919. DIE GODRONSCHE BASTARDE ZWISCHEN AEGILOPS- UND TRITICUMARTEN. VERERBUNG UND ZYTOLOGIE. *Ztschr. Induktive Abstam. u. Vererbungslehre* 20: [177]-240, illus.
- (5) BELLING, J.
1925. FRACTURE OF CHROMOSOMES IN RYE. *Jour. Heredity* 16: 360, illus.
- (6) BLEIER, H.
1928. GENETIK UND CYTOLOGIE TEILWEISE UND GANZ STERILER GETREIDE-BASTARDEN. *Bibliog. Genetica* 4: [321]-400.
- (7) ———
1928. ZYTOLOGISCHE UNTERSUCHUNGEN AN SELTENEN GETREIDE- UND RÜBENBASTARDEN. *Verhandl. 5th Internatl. Kong. Vererbungswiss Berlin* 2: [447]-452.
- (8) EMME, H. K.
1924. DIE RESULTATE DER ZYTOLOGISCHEN UNTERSUCHUNGEN EINIGER AEGILOPSARTEN. *Ztschr. Russ. Bot. Gesell.* 8: 193-197, illus. ((Referate) *Bot. Centbl.* 149: —. 1926.)
- (9) FERRAND,
1923. NOTE SUR LA CARYOCINÈSE DE SECALE CEREALE ET SUR UNE CAUSE D'ERREUR DANS LA NUMÉRATION DE SES CHROMOSOMES. *Bul. Soc. Roy. Bot. Belg.* 55: [186]-189, illus.
- (10) FLAKSBERGER, C. A.
1926. A CONTRIBUTION TO THE STUDY OF WILD MONOCOCCUM AND DICOCCUM AND THEIR PHYLOGENETIC CONNECTION WITH ONE ANOTHER AND WITH CULTIVATED VARIETIES. *Trudy Prikl. Bot. i. Selekt. (Bul. Appl. Bot. and Plant Breeding)* 16: 201-234, illus. [In Russian, résumé in English.]
- (11) GAINES, E. F., and AASE, H. C.
1926. A HAPLOID WHEAT PLANT. *Amer. Jour. Bot.* 13: 373-395, illus.
- (12) GOLIŃSKI, ST. J.
1893. EIN BEITRAG ZUR ENTWICKLUNGSGESCHICHTE DES ANDROECEUMS UND DES GYNACEUMS DER GRÄSER. *Bot. Centbl.* 55: 1-17, [65]-72, [129]-135, illus.
- (13) GOTOH, K.
1924. ÜBER DIE CHROMOSOMENZAHL VON SECALE CEREALE, L. *Bot. Mag. [Tokyo]* 38: 135-152, illus.
- (14) KAGAWA, F.
1927. CYTOLOGICAL STUDIES ON TRITICUM AND AEGILOPS. I. *Cellule* 37: 229-323, illus.
- (15) ———
1927. THE COMPARISON OF CHROMOSOMES AMONG DIFFERENT SPECIES IN TRITICUM. *Imp. Acad. Tokyo Proc.* 3: 304-306.
- (16) ———
1928. CYTOLOGICAL STUDIES ON TRITICUM AND AEGILOPS. II. ON THE GENUS CROSSES BETWEEN TRITICUM AND AEGILOPS. *Jap. Jour. Bot.* 4: 1-26, illus.

- (17) KIHARA, H.
1919. ÜBER CYTOLOGISCHE STUDIEN BEI EINIGEN GETREIDEARTEN. MITTEILUNG I. (SPEZIES-BASTARDE DES WEIZENS UND WEIZEN ROGGEN-BASTARDE). Bot. Mag. [Tokyo] 33: 17-38, illus.
- (18) ———
1921. ÜBER CYTOLOGISCHE STUDIEN BEI EINIGEN GETREIDEARTEN. MITTEILUNG III. ÜBER DIE SCHWANKUNGEN DER CHROMOSOMENZAHLEN BEI DEN SPEZIES BASTARDEN DER TRITICUMARTEN. Bot. Mag. [Tokyo] 35: 19-44, illus.
- (19) ———
1924. CYTOLOGISCHEN UND GENETISCHE STUDIEN BEI WICHTIGEN GETREIDEARTEN MIT BESONDERER RÜCKSICHT AUF DAS VERHALTEN DER CHROMOSOMEN UND DIE STERILITÄT IN DEN BASTARDEN. Mem. Col. Sci., Kyoto Imp. Univ. Ser. B 1: 1-200, illus.
- (20) ——— and NISHIYAMA, I.
1928. NEW ASPECTS OF CHROMOSOME BEHAVIOR IN POLLEN MOTHER-CELLS OF TRI-, TETRA- AND PENTAPLOID WHEAT HYBRIDS. Bot. Mag. [Tokyo] 42: 221-231, illus.
- (21) KOERNICKE, M.
1896. UNTERSUCHUNGEN ÜBER DIE ENTSTEHUNG UND ENTWICKLUNG DER SEXUAL-ORGANE VON TRITICUM, MIT BESONDERER BERÜCKSICHTIGUNG DER KERNTHEILUNGEN. Verhandl. Naturhist. Ver. Preuss. Rheinland 53: [149]-185, illus.
- (22) MELBURN, M. C., and THOMPSON, W. P.
1927. THE CYTOLOGY OF A TETRAPLOID WHEAT HYBRID. (TRITICUM SPELTA X T. MONOCOCCUM). Amer. Jour. Bot. 14: 327-333, illus.
- (23) MOL, W. DE.
1924. DE REDUCTIEDEELING BIJ EENIGE TRITICUM-SOORTEN. Genetica [The Hague] 6: [289]-329, illus.
- (24) NAKAO, M.
1911. CYTOLOGICAL STUDIES ON THE NUCLEAR DIVISION OF THE POLLEN MOTHER-CELLS OF SOME CEREALS AND THEIR HYBRIDS. Jour. Col. Agr., Tohoku Imp. Univ. 4: 173-190, illus.
- (25) NEMEC, B.
1910. DAS PROBLEM DER BEFRUCHTUNGSVORGÄNGE UND ANDERE CYTOLOGISCHE FRAGEN. 532 p., illus. Berlin.
- (26) NIKOLAEWA, A. G.
1922. ZUR CYTOLOGIE DER TRITICUMARTEN. (Referate) Ztschr. Induktive Abstam. u. Vererbungslehre 29: 208-209.
- (27) ———
1922-23. ÉTUDE CYTOLOGIQUE DU GENRE TRITICUM. Trudy Prikl. Bot. i Selekt. (Bul. Appl. Bot. and Plant Breeding) 13: 33-44, illus. [In Russian, French summary p. 42.]
- (28) ———
1924. [THE CYTOLOGY OF RYE-WHEAT HYBRIDS.] Nauk. Agron. Zhur. [Jour. Landw. Wiss.] 1: [570]-576, illus. [In Russian. Abstract in Bot. Abs. 15: 90.]
- (29) OVERTON, E.
1893. ÜBER DIE REDUKTION DER CHROMOSOMEN IN DEN KERNEN DER PFLANZEN. Vrtljschr. Naturf. Gesell. Zürich 38: 169-186.
- (30) PERCIVAL, J.
1921. THE WHEAT PLANT. A MONOGRAPH. 463 p., illus. London.
- (31) ———
1923. CHROMOSOME NUMBERS IN AEGILOPS. Nature 111: 810.
- (32) ———
1926. THE MORPHOLOGY OF SOME HYBRIDS OF AEGILOPS OVATA L. (FEMALE) X WHEATS (MALE). Jour. Genetics 17: 49-68, illus.
- (33) SAKAMURA, T.
1918. KÜRZE MITTEILUNG ÜBER DIE CHROMOSOMENZAHLEN UND DIE VERWANDTSCHAFTSVERHÄLTNISSE DER TRITICUM-ARTEN. Bot. Mag. [Tokyo] 32: 150-153.
- (34) SAPHENIN, A. A.
1928. PHYLOGENETIC INVESTIGATIONS OF THE VULGARE GROUP IN TRITICUM. Trudy Prikl. Bot. i Selekt. (Bul. Appl. Bot. and Plant Breeding) 19: 126-166, illus. [In Russian, summary in English p. 159-160.]

- (35) SAX, K.
1918. THE BEHAVIOR OF THE CHROMOSOMES IN FERTILIZATION. *Genetics* 3: [309]-327, illus.
- (36) ———
1921. CHROMOSOME RELATIONSHIPS IN WHEAT. *Science* (n. s.) 54: 413-415.
- (37) ———
1922. STERILITY IN WHEAT HYBRIDS. II. CHROMOSOME BEHAVIOR IN PARTIALLY STERILE HYBRIDS. *Genetics* 7: [513]-552, illus.
- (38) ———
1923. THE RELATION BETWEEN CHROMOSOME NUMBER, MORPHOLOGICAL CHARACTERS AND RUST RESISTANCE IN SEGREGATES OF PARTIALLY STERILE WHEAT HYBRIDS. *Genetics* 8: [301]-321, illus.
- (39) ———
1928. CHROMOSOME BEHAVIOR IN TRITICUM HYBRIDS. *Verhandl. 5th Internatl. Kong. Vererbungswiss.* Berlin 2: 1267-1284.
- (40) ——— and SAX, H. J.
1924. CHROMOSOME BEHAVIOR IN A GENUS CROSS. *Genetics* 9: [454]-464, illus.
- (41) SCHIEMANN, E.
1928. CHROMOSOMENZAHLEN IN DER GATTUNG AEGILOPS. *Ber. Deut. Bot. Gesell.* 46: 324-328, illus.
- (42) SOROKINA, O. N.
1928. ON THE CHROMOSOMES OF AEGILOPS SPECIES. *Trudy Prikl. Bot. i Selekt. (Bul. Appl. Bot. and Plant Breeding)* 19: 523-532, illus. [In Russian, summary in English].
- (43) SPILLMAN, W. J.
1911. A THEORY OF MENDELIAN PHENOMENA. *Amer. Breeders' Assoc.* 6: 78-90.
- (44) STOLZE, K. V.
1925. DIE CHROMOSOMENZAHLEN DER HAUPTSÄCHLICHSTEN GETREIDEARTEN NEBST ALLGEMEINEN BETRACHTUNGEN ÜBER CHROMOSOMEN, CHROMOSOMENZAHL UND CHROMOSOMENGRÖSSE IM PFLANZENREICH. *Genetica [The Hague]* 8: 1-171, illus.
- (45) THOMPSON, W. P.
1926. CHROMOSOME BEHAVIOR IN A TRIPLOID WHEAT HYBRID. *Jour. Genetics* 17: 43-48, illus.
- (46) ———
1926. CHROMOSOME BEHAVIOR IN A CROSS BETWEEN WHEAT AND RYE. *Genetics* 11: [317]-332, illus.
- (47) TSCHERMAK, E., and BLEIER, H.
1926. ÜBER FRUCHTBARE AEGILOPS-WEIZENBASTARDE. (BEISPIELE FÜR DIE ENTSTEHUNG NEUER ARTEN DURCH BASTARDIERUNG). *Ber. Deut. Bot. Gesell.* 44: 110-132, illus.
- (48) WATKINS, A. E.
1924-25. GENETIC AND CYTOLOGICAL STUDIES IN WHEAT. I-II. *Jour. Genetics* 14: [129]-171, illus. 1924. 15: [323]-366, illus. 1925.
- (49) WINGE, Ö.
1924. ZYTOLOGISCHE UNTERSUCHUNGEN ÜBER SPELTOIDE UND ANDERE MUTANTENÄHNLICHE ABERRANTEN BEIM WEIZEN. *Hereditas* 5: [241]-286, illus.
- (50) ZALENSKY, V. R., and DOROSHENKO, A. V.
1924-25. CYTOLOGICAL INVESTIGATION OF RYE-WHEAT HYBRIDS. *Trudy Prikl. Bot. i Selekt. (Bul. Appl. Bot. and Plant Breeding)* 14: [185]-210, illus. [In Russian, English summary p. 209-210].
- (51) ZHUKOVSKY, P.
1922-23. "PERSIAN WHEAT"—TRITICUM PERSICUM VAV. IN TRANSCAUCASIA. *Trudy Prikl. Bot. i Selekt. (Bul. Appl. Bot. and Plant Breeding)* 13: 45-55, illus. [In Russian, English summary p. 54-55].

INHERITANCE OF FUSARIUM RESISTANCE IN CABBAGE ¹

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INTRODUCTION

The diseases of plants caused by vascular Fusaria in many cases have been successfully controlled by the discovery or development, through selection and breeding, of host varieties resistant to attack. Resistant strains of cotton, flax, tomato, cabbage, and other plants are now used in areas where the respective crops are hazarded by this type of malady. In the main these forms have been developed from standard varieties, and in many cases highly resistant strains have been readily secured through mass selection. Comparatively little study has been made of the genetic behavior of Fusarium resistance in the plants mentioned. The investigations of Tisdale ² in the case of flax wilt indicated that resistance was controlled by multiple factors, but his studies were not extensive enough to warrant final conclusions.

In connection with selection for resistance within several varieties of cabbage which has been under way for a number of years, some attention has been given to this question. This paper is a report of such results as have a bearing upon the nature of inheritance of resistance to yellows caused by *Fusarium conglutinans* Wr.

DEFINITION OF THE PROBLEM

As already noted, ³ highly resistant strains of cabbage have been readily secured by selecting individuals that survive on badly infested soils. A few of such individuals grown in isolation but allowed to cross with one another ordinarily produced progenies showing increased resistance, and by repetition of this process once or twice, a highly resistant strain was usually secured. Complete resistance was not obtained under field conditions, although from the practical standpoint it was easy to maintain a high degree of resistance by selecting seed plants each year from the crop grown on infested soil where the small percentage of susceptible plants was eliminated.

In order to make a critical study of the inheritance of resistance it became necessary to procure, if possible, some lines homozygous for

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² TISDALE, W. H. FLAXWILT: A STUDY OF THE NATURE AND INHERITANCE OF WILT RESISTANCE. Jour. Agr. Research 11: 573-606, illus. 1917.

³ JONES, L. R., and GILMAN, J. C. THE CONTROL OF CABBAGE YELLOWS THROUGH DISEASE RESISTANCE. Wis. Agr. Expt. Sta. Research Bul. 38, 70 p., illus. 1915.

WALKER, J. C., and TISDALE, W. B. FUSARIUM RESISTANT CABBAGE. Wis. Agr. Expt. Sta. Research Bul. 48, 34 p., illus. 1920.

WALKER, J. C., MONTEITH, J., JR., and WELLMAN, F. L. DEVELOPMENT OF THREE MIDSEASON VARIETIES OF CABBAGE RESISTANT TO YELLOWS (*FUSARIUM CONGLUTINANS* WOLL.). Jour. Agr. Research 35: 785-809, illus. 1927.

resistance and others homozygous for susceptibility. The first step was to isolate selfed lines from mass-selected resistant lines, on the one hand, and from susceptible commercial varieties on the other. As has already been reported,⁴ it was thus possible to secure certain progenies which were completely resistant in the field and others of which the individuals were practically all susceptible. These were then used as parent stocks in crosses between resistant and susceptible lines. The behavior of these crosses and of subsequent generations derived from them, when exposed to the yellows organism, was then studied to determine, if possible, the law which governs the inheritance of resistance in cabbage.

Before discussing the details of experimentation it is important to consider briefly the symptoms of yellows.

SYMPTOMS OF YELLOWS

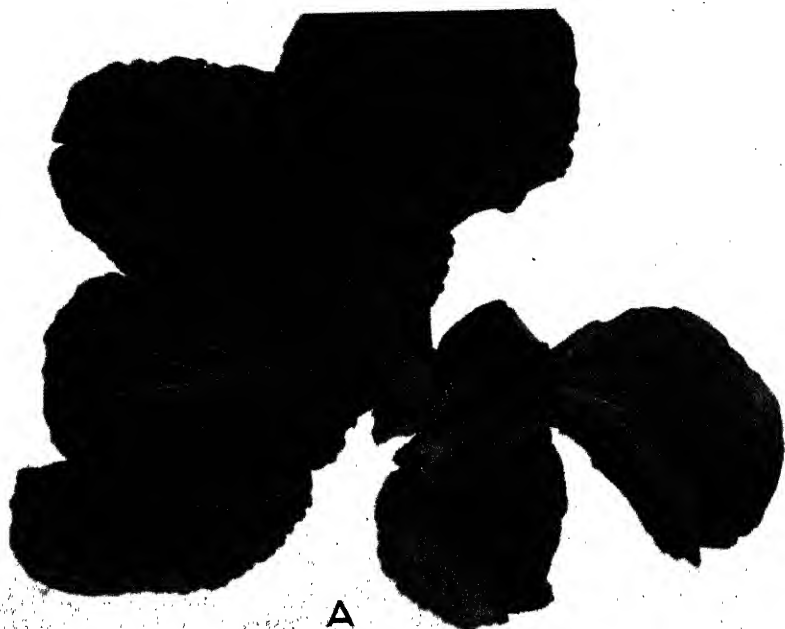
Yellows is most readily recognized by the characteristic yellowing of the foliage (pl. 1), which appears first in the parenchyma tissue between the veins. It may involve the whole leaf simultaneously, or it may be confined to the portion at one side of the midrib. In the latter case the reduced growth of the diseased portion commonly results in the unilateral development of the organ. Concomitant with or soon following the loss of normal green color, browning of the vascular elements in the diseased tissue can be seen upon cross sectioning. (Pl. 1, B.) Discolored xylem is usually associated with yellowed leaf parenchyma. The diseased parenchyma eventually dies, turns brownish, and becomes dry and brittle. The development of the abscission layer between petiole and stem is hastened, and premature defoliation results. By the time foliar symptoms appear, browning of the vascular system can be traced down the main stem into the taproot and sometimes into the lateral roots. Although the parasite enters through the young roots, there is little or no pathological effect visible upon the root system as a whole. Infection commonly occurs through rootlets on only one side of the underground part of the plant, and in progressing up the stem the fungus may be confined to a few bundles. Thus the unilateral development of the disease in the plant as a whole is common, and in such cases the individual leaves are often unilaterally affected also.

The disease, which is favored by rather high soil temperatures, may develop rather suddenly after the plants are exposed, or very gradually, depending upon the weather. Infected plants may be killed within a few weeks; but if unilateral infection occurs they may survive for a longer period. Gradations of severity are to be noted

⁴ WALKER, J. C. STUDIES UPON THE INHERITANCE OF FUSARIUM RESISTANCE IN CABBAGE. (Abstract) *Phytopathology* 16: 87. 1926.

EXPLANATORY LEGEND FOR PLATE 1

- A.—This plant was set in an infested field in southeastern Wisconsin during the last week of June. The photograph was taken about three weeks later. Note characteristic yellowing starting with the lower leaves. Unilateral development of infected leaves is common. The diseased parenchyma tissue later turns brownish in color and becomes dry and brittle. Affected leaves drop off prematurely and plants are commonly killed within a few weeks after the disease appears. Wilting of diseased plants seldom occurs, even in the middle of bright days.
- B.—A cabbage plant grown on infested soil along with A. The root system shows little or no injury although infection has taken place at the root tips. Browning of the fibrovascular bundles (chiefly in the tracheae of the xylem) is characteristic of the disease and follows closely upon the yellowing of the leaf parenchyma. The fungus is confined to the bundles until the plant has died when it may traverse the dead tissues and grow upon the surface.



A



B

CABBAGE YELLOWS

(For explanation legend, see opposite page)

PHOTOGRAPHED BY G. L. HARRIS

even up to cases where the symptoms appear on a single lower leaf which eventually drops off, while the plant heads normally without further development of the disease. The number of mild cases is of course greater during cool summers and when late infection is followed by only a short period of favorable temperature. Early, rapidly maturing varieties, well advanced before the soil temperature rises to a point favorable to the disease, usually exhibit fewer severe cases than those planted during the middle of the warm period. The long-season varieties, which even though planted early must normally grow throughout the period of warmest weather before they mature, are likely to show a higher percentage of severely infected individuals than the short-season varieties do. Aside from the diversity in severity of the disease due to purely environmental factors, hereditary differences between individuals of a variety or strain should not be overlooked. These will be given further consideration later in this paper.

Sudden loss of turgidity or wilting is not often the case, as in many herbaceous plants attacked by *Fusaria* through the xylem. In the greenhouse, when conditions are very favorable for the disease, it sometimes occurs, especially with young plants. In very young seedlings exposed to the disease, particularly in sterilized, artificially inoculated soil, the fungus sometimes invades the cortex at the base of the young hypocotyl, in which secondary thickening has scarcely started. This leads to a typical damping-off symptom involving sudden wilting and death. In the main, these last-described appearances involving loss of turgidity may be regarded as atypical and as usually occurring only under artificial environment.

METHODS OF EXPERIMENTATION

A discussion of the methods of selecting and handling plants for seed propagation has been given in a previous paper⁵ and need not be repeated in detail. In the main, plants were grown to seed in the greenhouse during the winter months, and in practically all cases where controlled pollination was practiced they were so handled. In some instances plants of the same genetic constitution, so far as the factors for resistance were concerned, were grown out of doors in an isolated place and allowed to be intercrossed by insects.

These studies were carried on in conjunction with a broader program which included the selection of resistant strains from a number of commercial varieties. Because of the limited greenhouse space it was necessary to handle a large number of seed plants in rather crowded quarters, and manipulation was carried out by a number of different workers. Branches on which flowers were being selfed or crossed were covered with paper bags. Even with reasonable care, however, a slight amount of contamination by foreign pollen was experienced. Where such pollen came from plants of distinctly different type usually the error could be readily detected in the subsequent trials, but this was not always possible. Instances will be cited, therefore, during the course of the discussion of the results where errors through pollen contamination or even through mechanical mixture of seeds of plants are suspected.

⁵WALKER, J. C., MONTEITH, J., Jr., and WELLMAN, F. L. Op. cit.

Tests for resistance or susceptibility discussed in this paper were usually carried out in the field upon thoroughly infested soil. Certain studies carried out subsequently under controlled conditions are discussed in another paper.⁶ The main testing ground was one used previously over a period of years during the selection of resistant varieties. It is located in the cabbage-growing area of southeastern Wisconsin (Kenosha County) and was selected in the beginning because of the thorough infestation of the soil with the yellows organism. Repeated cropping with cabbage has made it even more uniformly infested.

Earlier experience had shown that the most general infection under these local conditions occurred usually during the early part of July, especially if plants had been set a short time previously. The soil temperature, as a rule, is very favorable for disease during July and early August. After the middle of August the mean temperature gradually decreases. The seed was sown, therefore, in noninfested soil from about May 15 to June 1, and the plants for the most part were set in the trial plot during the first week of July. In 1927 the seed-bed soil was found to be somewhat infested, inasmuch as a few plants showed slight symptoms at transplanting. Although such plants were not eliminated at transplanting and were set out as they came, no appreciable difference in the results was noted.

After the appearance of the disease the trial plot was examined at frequent intervals and each plant showing symptoms was marked with a bamboo stake which was left until the end of the season. Each diseased plant was thus permanently labeled and could be recorded at any time later, even though it might in the meantime die and disappear or, on the other hand, recover from any external symptom. Since there were often differences in the severity of attack between individuals in a given progeny, and the significance of these differences was not completely understood, the diseased plants were classified at the end of the season into three categories: (1) Those dead or severely diseased; (2) those slightly diseased and continuing to show symptoms at the end of the season; and (3) those which at some time showed slight symptoms but which recovered from any external appearance before the end of the season.

As is usually the case in genetic studies of disease resistance, some difficulty was encountered in the differentiation of plants into two discontinuous classes; that is, healthy (resistant), and diseased (susceptible). It will be seen in the discussion, however, that the percentage of plants occupying the border line between the two classes was comparatively small. A large majority of the plants regarded as susceptible were either killed or severely diseased. A small percentage, averaging usually less than 10 per cent of the diseased plants, fell into the slight class. The plants that showed slight symptoms and recovered were relatively few and comprised on an average less than 3 per cent of the diseased class. There is some question whether the members of this last group should be classed as resistant or susceptible. An occasional plant in the resistant lines showed slight symptoms and recovered. On the other hand, three recovered plants from susceptible or segregating progenies yielded progenies which

⁶ WALKER, J. C., and SMITH, R. FACTORS INFLUENCING EXPRESSION OF FUSARIUM RESISTANCE IN CABBAGE. [Unpublished manuscript.]

reacted as did those from susceptible plants. It is possible that if the constitution of all the recovered plants had been studied by progeny tests some might have fallen into each class. Since this procedure was not feasible and the numbers were too small to affect the significance of the ratios, such plants were all placed in the susceptible class.

Further evidence of a well-defined distinction between the susceptible and resistant classes was gained through study of the two types when grown on infested soil under controlled conditions at a constant soil temperature of 24° C. As has been shown in detail in another paper,⁷ and by brief discussion herein, the plants of susceptible progenies succumbed promptly and completely at this temperature, whereas the resistant progenies withstood the disease perfectly. Furthermore, when segregating progenies were tested in this manner, the percentage of susceptible plants was practically identical with that in the field tests except that the disease appeared and progressed much more uniformly in the susceptible plants under controlled environmental conditions.

RESISTANT LINES FROM ALL HEAD EARLY AND GLORY OF ENKHUISEN VARIETIES

The first resistant line secured originated with a resistant plant selected in 1921 from an All Head Early progeny segregating into resistant and susceptible plants. This plant, 5-11, was grown to seed out of doors in complete isolation in 1922. The progeny, tested in 1923, showed 507 healthy and no diseased individuals. (Table 1.) Several individuals (5-21, 5-22, 5-24, 5-25, 5-27, 5-28, and 5-29) were selected from this progeny for seed production during the following winter. Certain of these plants were used as parents for resistant-susceptible crosses, while some flowers were self-pollinated and some sib crosses were made. The progenies from self-pollination and sib crosses were tested during 1924 and 1925. The results in Table 1 show that out of 260 second-generation plants only 3 were diseased. Of these, 1 died, 1 was slightly affected, and 1 recovered. Selected plants from the second generation were carried on to seed; selfs and sib crosses were made. With the exception of one selfed progeny (5-39s)⁸ and one plant which showed slight symptoms and recovered, all again showed freedom from disease. Plant 5-39 was evidently the result of a contamination. Its selfed progeny contained individuals entirely different in color of foliage and shape of head from the plants in the other progenies. It was, therefore, excluded from the totals.

This line was, with the exceptions noted, completely resistant to yellows and continued to remain so throughout three generations. Since the time of this selection numerous other individuals selected from All Head Early have yielded perfectly resistant progenies when selfed, and where they were continued they were repeatedly resistant in succeeding generations. Resistance remained stable in spite of

⁷ WALKER, J. C., and SMITH, R. Op. cit.

⁸ Throughout this paper the suffix "s" indicates that the progeny is the result of the self-pollination of a given plant. When a progeny is the result of a cross between two plants, e. g., 5-21X25 (Table 1), the number of the pistillate parent is given first. In some cases the blossom of the pistillate parent was not emasculated, but a brush was worked back and forth between blossoms of paired plants; and such progenies are therefore a mixture of plants resulting from selfing of the pistillate parent and from crossing with another staminate parent. Such a progeny is designated by the suffix B; e. g., H51-1X2B. (Table 7.)

the fact that in some cases there was a decided reduction in size and vigor of plants due to repeated selfing. There appears to be no correlation of resistance with pronounced vigor in these cases, as pointed out earlier with Wisconsin Hollander.⁹

TABLE 1.—Occurrence of yellows in progenies of the resistant line selected from the All Head Early variety of cabbage.

Generation	Progeny No.	Number of plants		Generation	Progeny No.	Number of plants	
		Diseased	Healthy			Diseased	Healthy
First.....	5-11s.....	0	507	Third.....	5-32s.....	0	16
Second.....	5-21s.....	a 2	92		5-32×34.....	0	43
	5-21×25.....	0	53		5-32×44.....	0	77
	5-22s.....	0	9		5-33s.....	0	32
	5-24s.....	0	7		5-34s.....	c 1	45
	5-25s.....	0	5		5-34×32.....	0	48
	5-27s.....	0	31		5-34×39.....	0	57
	5-28s.....	0	13		5-34×44.....	0	24
	5-28×27.....	0	38		5-38s.....	14	45
	5-29s.....	b 1	9		5-44s.....	0	20
					5-44×45.....	0	11
Total.....		3	257		5-45s.....	0	31
				Total.....		1	413
				Grand total.....		4	1,177

a 1 slight; 1 recovered.

b Severe.

c Recovered.

d Probably a result of contamination; results not included in totals.

The second resistant line used in making initial crosses was selected from Glory of Enkhuizen. Two plants from a partially resistant line were selfed. One of them yielded a segregating progeny and the other a completely resistant progeny. The homozygous plant, 35-12, was used as a resistant parent. Selections from the selfed progeny of this plant yielded completely resistant progenies, except one which, since it segregated into 25 per cent susceptible and 75 per cent resistant, was interpreted as a chance contamination during the self-pollination of 35-12.

SELECTION OF SUSCEPTIBLE PLANTS

As has been observed above, a large percentage of most commercial varieties of cabbage succumb to yellows when planted upon infested soil. A small number that survive yield, upon self-pollination or crossing within the group, progenies much higher in percentage of resistant individuals than the original variety from which they were selected. In order to secure susceptible individuals to use in crosses with resistant parents, plants of standard commercial varieties growing upon noninfested soil were selected. These were taken from three standard varieties, All Head Early, Glory of Enkhuizen, and Copenhagen Market. The results of progeny tests from self-pollination and crossing between certain individuals are given in Tables 2 to 4.

⁹ JONES, L. R., and GILMAN, J. C. *Op. cit.*

TABLE 2.—*Tests on yellows-infested soil of progenies secured from the All Head Early (commercial cabbage variety) seed plants grown the previous year on non-infested soil*

[H-1, H-2, H-3, H-6, H-11, and H-12 were used as susceptible parents in crosses with resistant parents, Tables 6 to 11]

Source of progeny (self or cross)	Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants diseased	
			Dead or severe	Slight	Re-covered	Diseased	Healthy
Selfed progenies	H-1s	1924	44	4	1	49	0
	H-2s	1925	17	2	0	19	6
	H-3s	1925	14	1	6	21	3
	H-4s	1924	0	0	2	2	4
	H-5s	1925	0	2	3	5	16
	H-6s	1924	8	0	0	8	2
	H-7s	1924	11	3	1	15	9
	H-11s	1924	11	1	5	17	4
	H-12s	1924	41	3	0	44	10
	H-2×1	1925	25	0	0	25	0
Crosses between plants yielding very susceptible selfed progenies.	H-3×2	1925	15	3	0	18	0
	H-6×1	1925	25	0	0	25	0
	H-6×1	1924	34	5	11	50	2
	H-11×12	1925	24	1	0	25	0
	H-11×12	1924	67	13	12	92	5
	H-12×11	1924	38	6	2	46	4
	H-1×4	1924	21	3	9	33	13
	H-1×4	1925	10	0	1	11	15
	H-4×1	1924	26	1	1	28	47
	H-2×4	1925	5	2	1	8	17
Crosses between plants yielding in one case susceptible and in the other segregating selfed progenies.	H-3×4	1925	8	2	2	12	13
	H-4×6	1924	36	4	15	55	24
	H-4×6	1925	9	3	0	12	12
	H-6×4	1924	8	5	6	19	21
	H-6×4	1925	13	3	0	16	9
	H-7×11	1924	17	9	3	29	61
	H-7×11	1925	6	1	2	9	16
	H-7×11	1924	21	1	0	22	34
	H-7×12	1924	23	2	1	26	19
	H-7×12	1925	12	0	0	12	12
		1926	20	5	2	27	29

TABLE 3.—*Tests on yellows-infested soil of progenies secured from Glory of Enkhvizen (commercial cabbage variety) seed plants grown the previous year on noninfested soil*

[G-7 and G-15 were used as susceptible parents in crosses with resistant parents, Tables 6 to 11]

Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants	
		Dead or severe	Slight	Recovered	Diseased	Healthy
G-2s	1925	18	5	2	25	0
G-5s	1925	8	0	1	9	1
G-7s	1924	5	1	0	6	0
G-15s	1924	7	0	0	7	0
G-4×12	1925	5	1	2	8	0
G-5×2	1925	23	1	11	35	1
G-7×5	1925	26	2	3	31	0
G-7×13	1924	95	8	10	113	7
G-13×12	1924	30	7	7	44	2
G-14×10	1925	8	1	1	9	0
G-14×12	1925	11	1	1	13	0
G-15×5	1924	28	0	0	28	1
	1925	18	0	0	18	1

TABLE 4.—Tests on yellows-infested soil of progenies secured from Copenhagen Market (commercial cabbage variety) seed plants grown the previous year on noninfested soil

Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants	
		Dead or severe	Slight	Recovered	Diseased	Healthy
C-4s.....	1926	0	1	0	1	11
	1927	14	0	0	14	18
C-4×9.....	1926	16	1	0	17	17
	1926	22	2	0	24	4
C-6s.....	1927	14	0	0	14	0
	1926	27	5	0	32	0
C-7s.....	1927	39	0	0	39	0
	1926	32	6	3	41	2
C-7×6.....	1927	18	1	2	21	1
	1926	3	0	0	3	0
C-9s.....	1926	5	1	0	6	11
C-12s.....	1927	7	0	5	12	4
C-12×H-24.....	1926	6	2	1	9	0
	1926	2	0	0	2	4
C-13s.....	1927	4	0	0	4	29
	1926	0	2	2	4	9
C-13×H-27.....	1927	4	0	6	10	15
	1926	3	16	7	26	0
C-14s.....	1927	21	0	0	21	0
C-15s.....	1927	2	0	13	15	1
C-17s.....	1927					

In the All Head Early plants an examination of the data (Table 2) shows that plants H-1,¹⁰ H-2, H-3, H-6, H-11, and H-12 when selfed, or crossed with one another, yielded progenies quite susceptible to yellows. Plants H-4, H-5, and H-7 yielded selfed progenies with considerable percentages of resistant plants, and in the progenies from crosses with others in the first group resistant plants were about as numerous as susceptible ones. In the Glory of Enkhuizen group (Table 3) all plants yielded very susceptible progenies whether selfed or crossed with others in the group. In the Copenhagen Market group (Table 4), C-4 and C-13 yielded selfed and hybrid progenies with a high percentage of resistant plants, while the others in the group, except C-12, produced very susceptible families. C-12 might be classed with C-4 and C-13 were it not for the fact that when crossed with a susceptible individual of the All Head Early variety (H-24), a completely susceptible F₁ progeny resulted. As plant C-12 stood close to plant C-13 in the greenhouse it is possible that some pollen contamination occurred while the bags were removed for selfing.

It may be well to consider the behavior of these progenies from susceptible varieties in the light of data which were subsequently secured and presented in this paper and which seem to show that resistance in the cases studied is governed by a single dominant factor. It is suggested that in the picking of plants at random from a commercial variety growing upon yellows-free soil one may expect occasionally to secure a plant which when selfed yields a progeny of much higher resistance than the average of the lot from which it was chosen. On the single-factor basis such an individual may be heterozygous or homozygous for resistance. None of those encoun-

¹⁰ Throughout this paper H denotes progeny developed from the All Head Early variety, C that from Copenhagen Market, G that from Glory of Enkhuizen, and WC that from wild cabbage.

tered in the selections just described were homozygous for resistance. Out of the 31 plants studied, coming from 3 varieties, 5 individuals (H-4, H-5, H-7, C-4, and C-13) seem to have been heterozygous for resistance. This is indicated by the behavior of their selfed progenies and shown more conclusively in the large populations of the hybrids resulting from the crossing of these individuals with others in the series which seemed to be homozygous for susceptibility.

In the case of the remaining plants of the series (excepting C-12) a large share of those in selfed progenies and of the hybrids from crosses within the group were diseased. It is to be noted, however, that all plants were not equally affected. A majority were severely diseased, while a smaller number were slightly affected and a few recovered. A completely satisfactory explanation for this variation can not be given. Under the conditions of the trials it may reasonably be expected, however, that unevenness of distribution of the pathogene in the soil may account for it in part. Some light on the question might be obtained by growing such individuals to seed and testing the progenies. However, little success has been had in carrying mildly affected plants to maturity of seed. Three plants in the recovered class have so far been grown to seed and their progenies tested. All of these yielded progenies which reacted similarly to those from plants known to be susceptible. There is thus no evidence yet at hand to indicate that the plants in this class are necessarily heterozygous or homozygous for resistance, although a study of a larger number might show some to belong to one or another of these two classes.

Two possible explanations may be offered to account for the fact that some plants remained healthy in these very susceptible progenies. Some of them may have accidentally escaped infection, while others may have undergone chance contamination with foreign pollen from heterozygous or homozygous resistant plants. Two surviving healthy plants from H-12 \times 11 were grown to seed. The selfed progenies of each indicated that both of these survivors were heterozygous. One yielded 13 healthy to 7 diseased plants while the other yielded 12 resistant to 4 diseased. A portion of the C-12s progeny was grown on yellows-free soil, and 5 individuals were carried on to seed. Of these, 3 proved to be homozygous for susceptibility and 2 were clearly heterozygous. It is quite likely that in both these progenies, H-12 \times 11 and C-12s, there had been slight contamination by foreign pollen.

Further light on the inheritance of susceptibility was secured by the continuation of certain of the lines. Two plants from progeny H-1s grown on yellows-free soil were grown to seed, and the selfed progenies were tested on infested soil. One of these tested in 1925 and 1926 yielded 99 diseased and 14 healthy plants; the other tested during the same year yielded 72 diseased and 2 healthy. A number of crosses were made in which the staminate and pistillate parents came from different varieties. Some of these are already recorded in Table 4. Three others, with their consequent behavior on infested soil, are given in Table 5. A certain number of the F₁ plants from H-12 \times G-15 and H-16 \times C-9 were grown on yellows-free soil and carried through to the F₂ and F₃ generations. In general the percentage of healthy plants that survived when the F₂ and F₃ progenies were tested was low. (Table 5.) In a few cases in the HC series (e. g.,

HC-11×14B) the number of healthy plants was unusually high, but when this progeny was tested the following season no plants survived, indicating that in the first trial the healthy plants merely escaped infection.

TABLE 5.—Occurrence of yellows in hybrid progenies from crosses between susceptible plants of Glory of Enkhuizen (G), All Head Early (H), and Copenhagen Market (C) varieties of cabbage, with F_2 and F_3 selections made from some of the crosses, when grown upon yellows-infested soil

Cross	F ₁ plants		F ₂ plants				F ₃ plants		
	Num-ber dis-eased	Num-ber healthy	Progeny No.	Year tested	Num-ber diseased	Num-ber healthy	Progeny No.	Num-ber diseased	Num-ber healthy
G-7×H-11	64	2	HG-1s	1927	11	0	HG-101×102B	22	2
							HG-102×101B	11	2
							HG-103×105B	23	1
H-12×G-15	80	0	HG-2s	1927	27	3			
			HG-3s	1927	19	1			
			HG-4s	1927	23	2			
			HG-5s	1927	24	1			
			HC-2×HC-1	1927	24	0			
				1928	61	0			
			HC-2s	1927	42	1			
			HC-3×19B	1927	22	2			
				1927	18	4			
			HC-4s	1927	21	1			
				1928	25	0			
			HC-5s	1927	26	0			
			HC-6×13B	1927	26	0			
			HC-6s	1927	28	0			
			HC-7s	1927	13	2			
				1927	38	2			
			HC-7×18B	1928	50	0			
				1927	24	1			
			HC-10×6B	1928	25	0			
			HC-11s	1927	17	8			
				1927	14	11			
			HC-11×14B	1928	15	0			
				1927	16	9			
			HC-12s	1928	20	5			
			HC-13×5B	1927	25	0			
				1927	21	4			
			HC-14s	1928	19	0			
				1927	22	3			
			HC-14×11B	1928	21	0			
				1927	44	4			
			HC-15s	1928	21	0			
				1927	15	1			
			HC-16s	1928	26	1			
H-16×C-9	159	3							

Later in this investigation greenhouse tests were made with a number of these progenies, the purpose being to subject the plants to conditions which would more certainly produce uniform infection. Both naturally infested soil from the test plot and soil that had been sterilized and inoculated with a pure culture of the causal organism were used. After the plants had recovered from transplanting the containers were placed in soil temperature tanks at 23°-24° C., where, as previous work had shown, homozygous resistant plants and heterozygous plants remain healthy, while homozygous susceptible plants succumb promptly. When progenies HC-4s, HC-7×18B, HC-11×14B, HC-14s, HC-14×11B, HC-15s, HC-16s, HG-101×102B, and HG-103×105B were submitted to this test all plants became diseased within one week after the cans were placed in the tanks. This would seem to indicate that a great many of the healthy plants that survived in the field tests had escaped infection. Whether

the moderate development of disease in certain of the susceptible plants was due to chance escape of part of the root system from infection, to variations in soil environment, or to hereditary modifying factors remains for the present unsettled. It is probable that each of the three is a potential cause of such variation.

To summarize, it may be stated that most plants selected from the three commercial varieties gave progenies which were very susceptible to the disease. A small number yielded progenies that were much more resistant to yellows than the rest, and these were probably heterozygous plants of the same order as many of those that survive when commercial varieties are planted upon infested soil. The others are regarded for the present as showing evidence of being homozygous for susceptibility.

INHERITANCE OF RESISTANCE

IN ALL HEAD EARLY AND GLORY OF ENKHUIZEN VARIETIES

F₁ GENERATION

Crosses of resistant plants, 5-21 to 5-29 (derived from All Head Early) and 35-12 (derived from Glory of Enkhuizen) with the susceptible plants H-1, H-2, H-3, H-4, H-5, H-6, H-7, H-11, H-12, G-5, G-7, and G-15 were made. Since H-4, H-5, and H-7 were later shown to be distinctly heterozygous, crosses into which they entered are dropped from further consideration here. The initial hybrid progenies were first tested in 1924, and many were repeated in the trials of the following seasons up to and including 1927. A summary of the records as to their behavior when planted on infected soil is given in Table 6. The dominance of the resistant character is very consistent throughout. Out of a total of 2,773 plants, 24 showed the disease; of these 5 recovered, 1 was slightly affected, and 18 were severely diseased. In several instances the susceptible plants did not appear consistently when the progeny was tested during several seasons. It is quite possible that they resulted from a mechanical mixture of seed or of plants, or they may have been caused by an occasional accidental self-pollination during the process of crossing. It is to be noted in the latter connection that the diseased plants appeared, in all cases but two, where the susceptible plant was used as the pistillate parent, and in these two exceptions the plants showed only slight symptoms and later entirely recovered from any external signs of yellows. The behavior of the resistant and susceptible lines and the F₁ hybrid in the 1925 trial plot is illustrated in Figure 1. In the center is the susceptible progeny, H-3×2 (Table 2), in which all plants were diseased. At the right is the resistant progeny, 5-28×27 (Table 1), which was entirely healthy. At the left is the hybrid progeny, H-1×5-23 (Table 6), which was equal in resistance to the homozygous resistant progeny, 5-28×27.



FIGURE 1.—Comparison of homozygous resistant, homozygous susceptible, and F_1 hybrid progenies on the naturally infested field-trial plot in Kenosha County, Wis., in 1925. A, Cross between susceptible and resistant plants (H-1×5-23, Table 6); all plants remained healthy. B, Susceptible progeny (H-3×2, Table 2); all plants were diseased. C, Resistant progeny (5-28×27, Table 1); all plants were healthy.

TABLE 6.—Occurrence of yellows in F_1 hybrid progenies resulting from crosses between homozygous resistant and homozygous susceptible cabbage plants when grown in the field upon infested soil

Cross	Year tested	Number of plants		Cross	Year tested	Number of plants	
		Diseased	Healthy			Diseased	Healthy
H-1×5-23-----	1924	0	51	H-6×5-23-----	1924	0	42
	1925	0	26		1925	0	25
	1926	0	62		1924	0	43
	1924	a 4	59		1925	0	25
H-1×5-24-----	1925	b 2	67	H-6×5-25-----	1924	0	58
	1926	0	31		1924	0	14
5-24×H-1-----	1924	0	15	H-11×5-21-----	1924	0	63
	1924	0	56		5-21×H-11-----	1924	e 1
H-1×5-25-----	1925	0	107	H-11×5-25-----	1924	d 1	129
	1926	0	47		5-25×H-11-----	1924	e 1
5-25×H-1-----	1924	0	52	H-11×5-26-----	1924	0	70
	1924	1	77		1925	0	28
H-1×5-27-----	1925	0	49	H-12×5-21-----	1924	0	82
	1926	0	45		1925	0	73
5-27×H-1-----	1924	0	12	5-21×H-12-----	1924	0	42
	1924	0	79		H-12×5-24-----	1924	0
H-1×5-28-----	1925	0	29	H-12×5-25-----	1925	b 1	54
	1924	b 1	59		5-25×H-12-----	1924	0
H-1×5-30-----	1925	0	26	H-12×5-28-----	1924	b 1	58
	1925	0	51		G-5×5-24-----	1925	0
H-2×5-23-----	1925	0	18	G-5×5-25-----	1925	0	25
H-2×5-24-----	1924	0	30	G-5×5-26-----	1925	b 4	22
H-2×5-27-----	1925	0	35	G-7×35-12-----	1924	0	50
H-2×5-28-----	1925	0	20	G-15×5-21-----	1925	b 2	24
5-28×H-2-----	1926	0	25	G-15×5-24-----	1924	e 1	52
H-2×5-30-----	1925	0	31		1925	d 1	32
H-3×5-23-----	1925	0	24	G-15×5-25-----	1924	0	51
H-3×5-24-----	1925	b 1	15		1925	0	71
H-3×5-27-----	1925	0	28	G-15×5-26-----	1924	e 2	53
H-3×5-28-----	1925	0	24		1925	0	24
H-3×5-30-----	1925	0	25	Total-----		24	2,749

F₂ GENERATION

Plants were selected from a number of progenies from the F₁ hybrid tests in 1924 and from others in 1925. Those from the following progenies were grown to seed in the greenhouse: H-1 × 5-24, 5-24 × H-1, H-1 × 5-25, 5-25 × H-1, G-15 × 5-24, and G-15 × 5-25. Seed from self-pollination was secured whenever possible. Inasmuch as the number of seeds from selfing is often very low, a larger quantity of seed was insured by making sib crosses between plants of the same parentage. These were secured by merely working a camel's-hair brush over blossoms of paired plants, or in some cases a number of heads from a single cross were carried over winter and planted in an isolated group out of doors the following summer. In cases like the latter the seed from all the plants of the lot was usually mixed. The F₂ progeny tests were made in the trial plot described before, care being taken to select as uniformly infested soil as possible and to plant at times favorable for infection. As was noted in the susceptible lines, all plants that became diseased did not show equally severe infection. Records were therefore kept according to the arbitrary classification of diseased plants explained above. The first F₂ progeny trials were made in 1925 and were continued during 1926, 1927, and 1928.

In Table 7 are given the results with the F₂ progenies from the reciprocal crosses, H-1 × 5-24 and 5-24 × H-1. The large majority of the diseased plants fell into the severe class. Variation in the rate of development of the disease in the susceptible group was therefore no greater, in fact it was less, than in the selfed progeny of the susceptible parent shown in Table 2. It is evident that every progeny segregated as a monohybrid, yielding close to 25 per cent diseased plants. The deviation from the theoretical 3 to 1 ratio was usually negligible, while in practically every case where it was more than three times the probable error repetition in another year gave a reasonably close fit. The progenies from sib crosses reacted essentially the same as those from selfed blossoms. One selfed progeny (H5I-4s)¹¹ showed a poor fit in each of three years. It should be noted, however, that in 1925 and 1927 the number of susceptible plants was higher, while in 1926 it was lower, than the expected. This irregularity may have been due in part to variation in the infestation of the plot, although this is the only progeny which gave such a poor fit consistently. In the sib cross between this plant and another (H5I-5, segregation in the selfed progeny of which was a reasonably close fit) the deviation was less than twice the probable error.

¹¹ Throughout this paper the use of I in the number designates an F₁ plant from a resistant-susceptible cross or an F₂ progeny from such a plant; i. e., the F₁ hybrid plants selected for seed propagation are each given a number including the letter I. Thus the F₂ progenies therefrom are designated by the parent plant number with the appropriate suffix to indicate whether the seed was secured by self-pollination or by crossing. In this instance H5I-4 is the number given the F₁ hybrid plant. H5I-4s is the number of the F₂ self-progeny derived from that plant.

TABLE 7.—Occurrence of yellows in F_2 cabbage progenies from the reciprocal crosses, $H-1 \times 5-24$ and $5-24 \times H-1$ and $H-1 \times 5-25$ and $5-25 \times H-1$ (Table 6), when grown in the field upon infested soil

Original cross No. and kind of cross	Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. P. E.
			Dead or severe	Slight	Recovered	Diseased	Healthy			
$H-1 \times 5-24$: Self-----	H5I-1s-----	1925	12	0	0	12	37	0.3	2.0	0.2
		1925	17	2	0	19	40	4.3	2.2	2.0
	H5I-2s-----	1926	34	1	0	35	82	5.8	3.2	1.8
		1925	7	1	1	9	27	0	1.8	0
	H5I-3s-----	1926	20	0	1	21	73	2.5	2.8	.9
		1925	24	0	2	26	39	9.8	2.4	4.1
	H5I-4s-----	1926	9	1	0	10	82	13.0	2.8	4.6
		1927	10	1	0	11	15	4.5	1.5	3.0
	H5I-5s-----	1925	11	0	3	14	43	.3	2.2	.1
		1926	20	3	0	23	57	3.0	2.6	1.2
	H5I-6s-----	1926	24	3	0	27	91	2.5	3.2	.8
		1927	22	3	0	25	34	10.3	2.2	4.7
Total-----			210	15	7	232	620	19.0	8.5	2.2
Sib-----	H5I-1 \times 2B----	1926	4	0	0	4	17	1.3	1.3	1.0
	H5I-2 \times 1B----	1925	10	1	0	11	31	.5	1.9	.3
	H5I-3 \times 1B----	1926	20	0	0	20	65	1.3	2.7	.5
	H5I-4 \times 5B----	1926	19	1	1	21	83	5.0	3.0	1.7
	H5I-5 \times 4B----	1925	12	0	2	14	40	.5	2.2	.2
Total-----			65	2	3	70	236	6.5	5.1	1.3
$5-24 \times H-1$: Self-----	5HI-4s-----	1925	7	0	0	7	40	4.8	2.0	2.4
		1926	33	1	0	34	69	8.3	3.0	2.8
		1925	18	4	0	22	67	.3	2.8	.1
	5HI-6s-----	1926	23	1	2	26	97	4.8	3.2	1.5
		1927	11	0	0	11	24	2.3	1.7	1.4
Total-----			92	6	2	100	297	.8	5.8	.1
Sib-----	5HI-2 \times 5B----	1925	7	5	0	12	46	2.5	2.2	1.1
	5HI-6 \times 4B----	1925	15	2	0	17	46	1.3	2.3	.6
		1926	24	1	0	25	83	2.0	3.0	.7
Total-----			46	8	0	54	175	3.3	4.4	.8
Grand total-----			413	31	12	456	1,328	10.0	12.3	.8
Grand total calculated on 1:3 ratio-----						446	1,338			
$H-1 \times 5-25$: Self-----	H5I-12s-----	1925	4	2	0	6	49	7.8	2.2	3.5
		1926	20	0	3	23	83	3.5	3.0	1.2
		1927	0	2	2	4	22	2.5	1.5	1.7
	H5I-14s-----	1925	17	3	2	22	45	5.3	2.4	2.2
		1926	16	0	0	16	44	1.0	2.3	.4
Total-----			57	7	7	71	243	7.5	5.2	1.4
Sib-----	H5I-12 \times 14B----	1925	7	0	0	7	41	5.0	2.0	2.5
		1926	23	1	0	24	76	1.0	2.9	.3
	H5I-14 \times 12B----	1925	6	0	0	6	16	.5	1.4	.4
		1926	15	0	1	16	33	3.8	2.0	1.9
Total-----			51	1	1	53	166	1.8	4.3	.4
$5-25 \times H-1$: Self-----	5HI-12s-----	1925	16	3	4	23	59	2.5	2.6	1.0
		1926	7	0	0	7	33	3.0	1.9	1.6
	5HI-14s-----	1925	5	0	0	5	20	1.8	1.5	.9
Total-----			28	3	4	35	112	1.8	3.5	.5
Sib-----	5HI-11 \times 14B----	1925	10	1	0	11	31	.5	1.9	.3
	5HI-14 \times 11B----	1926	15	0	0	15	70	6.3	2.7	2.3
	5HI-12 \times 13B----	1925	7	1	0	8	32	2.0	1.9	1.1
		1926	17	4	0	21	63	0	2.7	0
Total-----			49	6	0	55	196	7.8	4.6	1.7
Grand total-----			185	17	12	214	717	18.8	8.9	2.1
Grand total calculated on 1:3 ratio-----						233	698			

TABLE 8.—Occurrence of yellows in F_2 cabbage progenies from the crosses, $G-15 \times 5-24$, $G-15 \times 5-25$, and $G-7 \times 35-12$ (Table 6), when grown in the field upon infested soil

Original cross No. and kind of cross	Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. P. E.
			Dead or severe	Slight	Recovered	Diseased	Healthy			
$G-15 \times 5-24$: Self.....	(G5I-1s.....	1925	5	0	0	5	34	4.8	1.8	2.7
	(G5I-14s.....	1926	10	0	0	10	37	1.8	2.0	.9
	(G5I-16s.....	1927	21	0	0	21	27	9.0	2.0	4.5
	(G5I-18s.....	1926	11	3	0	14	33	2.3	2.0	1.2
	(G5I-17s.....	1926	11	2	0	13	34	1.3	2.0	.7
	(G5I-21s.....	1927	11	0	0	11	46	3.3	2.2	1.5
Total.....			69	5	0	74	211	2.8	4.9	.6
$G-15 \times 5-25$: Self.....	(G5I-6s.....	1925	31	2	2	35	93	3.0	3.3	.9
	(G5I-23s.....	1927	15	0	0	15	35	2.5	2.1	1.2
	(G5I-28s.....	1926	10	0	0	10	39	2.3	2.0	1.2
	Total.....		56	2	2	60	167	3.3	4.4	.8
Sib.....	(G5I-4×5B.....	1925	7	0	0	7	41	5.0	2.0	2.5
	(G5I-5×4B.....	1926	15	0	0	15	34	2.8	2.0	1.4
	(G5I-5×4B.....	1925	27	2	1	30	66	6.0	2.9	2.1
	(G5I-5×4B.....	1926	11	0	0	11	36	.8	2.0	.4
Total.....			60	2	1	63	177	3.0	4.5	.7
$G-7 \times 35-12$: Self.....	(G35I-7s.....	1925	5	0	0	5	15	.0	1.3	0
	(G35I-10.....	1926	3	0	0	3	12	.8	1.1	.7
	(G35I-11.....	1926	40	0	0	40	108	3.0	3.6	.8
	(G35I-12.....	1926	27	2	1	30	80	2.5	3.1	.8
	(G35I-13.....	1926	23	7	0	30	77	3.3	3.0	1.1
	(G35I-14.....	1926	34	5	2	41	127	1.0	3.8	.3
	(G35I-15.....	1927	12	3	0	15	39	1.5	2.2	.7
	(G35I-16.....	1926	57	0	0	57	130	10.3	4.0	2.6
	(G35I-17.....	1927	17	1	0	18	31	5.8	2.0	2.9
Total.....			218	18	3	239	619	24.5	8.6	2.8
Grand total.....			403	27	6	436	1,174	33.5	11.7	2.9
Grand total calculated on 1:3 ratio.						402	1,208			

The F_2 progeny tests from the reciprocal crosses $H-1 \times 5-25$ and $5-25 \times H-1$, are summarized in Table 7. The segregation is again consistently that to be expected on the monohybrid basis.

The progenies discussed so far are from crosses where the resistant and susceptible parents were both derived originally from All Head Early. In Table 8 are the results with F_2 progenies from two crosses wherein a Glory of Enkhuizen plant ($G-15$) was used as the susceptible parent and All Head Early plants ($5-24$ and $5-25$) were used as resistant parents. Results with progenies from a cross between a susceptible and a resistant plant, both derived from Glory of Enkhuizen ($G-7 \times 35-12$), are also included in this table. In the last case only one plant from a selfed plant was secured, but a number of F_1 plants were carried through winter storage and set out of doors the following spring in an isolated location. Cross-fertilization by bees went on within the group, and the seed from each plant was saved separately. These progenies ($G35I-10$ to 14) were also tested and the results are included in Table 8. In all the progenies from these three crosses there is close conformity to simple segregation into 3 resistant to 1 susceptible. The comparatively small proportion of

diseased plants falling within the slight and recovered groups is also worthy of notice.

Many more plants were saved from the 1924 tests of F_1 progenies than could be grown to seed the following winter in the greenhouse. The surplus plants were therefore stored during the winter, and the following spring plants from the same cross or closely related crosses were grouped together for seed growing in isolated areas out of doors during the summer of 1925. The seed from the individual plants within each lot was combined. These strains were tested during 1926, 1927, and 1928. The results are assembled in Table 9. Although a wider range of susceptible and resistant parents are included in the crosses represented here, it is evident that the F_2 distributions are in as close agreement with the expectation on the monohybrid basis as those recorded in the two previous tables.

The data concerning the tests of F_2 progenies coming from resistant-susceptible crosses of All Head Early and Glory of Enkhuizen plants and given in detail in Tables 7 to 9 are summarized in Table 10. The total figures for each of four consecutive seasons show very close fits, indicating that the conditions under which the trials were made were perhaps as uniform as could be reasonably expected under field conditions. Variation within the susceptible group as to severity of disease was no greater than that previously described in the susceptible lines.

TABLE 9.—Occurrence of yellows in F_2 cabbage progenies from several groups of F_1 plants in the field when grown upon infested soil

[Plants from a single cross or closely related crosses were grown in isolation, natural cross pollination with each group being permitted; the seed from all plants within a single group was combined into one lot]

Original cross	F_2 progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. P. E.
			Dead or severe	Slight	Recovered	Diseased	Healthy			
H-1×5-25-----	H5IA-----	1926	25	2	0	27	70	2.8	2.9	1.0
		1927	11	3	1	15	34	2.8	2.0	1.4
		1928	17	4	0	21	75	3.0	2.9	1.0
5-25×H-1-----	5HIA-----	1926	28	5	2	35	87	4.5	3.2	1.4
		1927	38	7	0	45	157	5.5	4.2	1.3
H-6×5-23-----	H5IB-----	1926	16	0	0	16	32	4.0	2.0	2.0
H-6×5-24-----		1927	7	0	2	9	38	2.8	2.0	1.4
H-6×5-27-----	H5IC-----	1926	8	1	0	9	21	1.5	1.6	.9
H-11×5-25-----		1927	8	0	0	8	13	2.8	1.3	2.2
H-12×5-21-----	H5ID-----	1926	62	13	5	80	223	4.3	5.1	.8
H-12×5-24-----		1927	53	10	1	64	230	9.5	5.0	1.9
H-12×5-25-----		1928	87	15	0	102	284	5.5	5.7	1.0
H-12×5-26-----	G5IA-----	1926	9	0	0	9	13	3.5	1.3	2.7
G-15×5-25-----		1928	6	1	0	7	38	4.3	2.0	2.2
G-15×5-24-----	G5IB-----	1926	8	1	0	9	41	3.5	2.1	1.7
G-15×5-25-----		1928	40	0	0	40	97	5.8	3.4	1.7
G-7×35-12-----	G35IA-----	1928	8	1	0	9	41	3.5	2.1	1.7
G-15×5-24-----	G5IC-----	1928	40	0	0	40	97	5.8	3.4	1.7
Total-----			423	62	11	496	1,453	8.8	12.9	.7
Total calculated on 1:3 ratio.						487	1,462			

TABLE 10.—Summary of segregation of F_2 cabbage progenies from crosses between resistant and susceptible lines of All Head Early and Glory of Enkhuizen varieties when grown upon infested soil

Cross	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. P. E.
	Dead or severe	Slight	Recovered	Diseased	Healthy			
H-1×5-24.....	275	17	10	302	856	12.5	9.9	1.3
5-24×H-1.....	133	14	2	154	472	2.5	7.3	.3
H-1×5-25.....	108	8	8	124	400	9.3	6.7	1.4
5-25×H-1.....	77	9	4	90	308	9.5	5.8	1.6
G-15×5-24.....	69	5	0	74	211	2.8	4.9	.6
G-15×5-25.....	116	4	3	123	344	6.3	6.3	1.0
G-7×35-12.....	218	18	3	239	619	24.5	8.6	2.8
Miscellaneous.....	423	62	11	496	1,453	8.8	12.9	.7
Total for 1925.....	287	29	17	333	998	.3	10.6	.03
Total for 1926.....	783	57	18	858	2,491	20.8	16.9	1.2
Total for 1927.....	236	30	6	272	745	17.8	9.3	1.9
Total for 1928.....	118	21	0	139	438	5.3	7.0	.8
Grand total.....	1,424	137	41	1,602	4,672	33.5	23.1	1.5
Grand total calculated on 1:3 ratio.....	-----	-----	-----	1,569	4,705	-----	-----	-----

CROSSES BETWEEN F_1 HYBRIDS AND SUSCEPTIBLE PLANTS

A few back crosses were made between F_1 hybrids and susceptible plants. The occurrence of yellows in these progenies when tested upon infested soil is recorded in Table 11. In practically all cases there was a reasonably close fit to the 1:1 segregation expected. The behavior of the F_1 , F_2 , and back-crossed progenies in the 1927 trial plot is shown in Figure 2.

TABLE 11.—Occurrence of yellows in back crosses of F_1 cabbage hybrids (resistant and susceptible) with susceptible individuals

Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. P. E.
		Dead or severe	Slight	Recovered	Diseased	Healthy			
H5I-1×C-1.....	1925	10	0	0	10	20	5.0	1.9	2.6
H5I-1×H-35.....	1925	8	0	0	8	8	0	1.4	0
	1925	25	2	0	27	25	1.0	2.4	.4
H5I-2×C-1.....	1926	20	4	0	24	50	13.0	2.9	4.5
	1925	21	4	1	26	23	1.5	2.4	.6
H5I-2×H-35.....	1925	24	3	0	27	24	1.5	2.4	.6
H5I-12×C-1.....	1925	12	4	5	21	33	6.0	3.2	4.1
	1926	30	3	0	33	59	13.0	2.4	3.3
H5I-12×H-35.....	1926	13	4	0	17	33	8.0	2.4	3.3
	1927	15	6	0	21	20	.5	2.2	.2
H5I-14×H-35.....	1925	14	0	1	15	25	5.0	2.1	2.4
5HI-5×H-1.....	1925	13	3	0	16	11	2.5	1.8	1.4
	1925	29	2	1	32	17	7.5	2.4	3.1
5HI-5×C-1.....	1926	8	2	0	10	14	2.0	1.7	1.2
	1925	23	0	0	23	14	4.5	2.1	2.1
5HI-11×C-1.....	1925	17	0	0	17	25	4.0	2.2	1.8
5HI-12×C-2.....	1925	9	3	2	14	19	2.5	1.9	1.3
	1926	11	2	0	13	13	0	1.7	0
C-12×G5I-13.....	1926	19	0	0	19	16	1.5	2.0	.8
	1926	15	8	2	25	24	.5	2.4	.2
G5I-14×C-12.....	1927	40	0	0	40	37	1.5	3.0	.5
	1926	20	0	0	20	31	5.5	2.4	2.3
C-12×G5I-14.....	1927	5	0	0	5	6	.5	1.1	.5
	1926	17	5	0	22	25	1.5	2.3	.7
C-12×G5I-28.....	1927	16	0	0	16	16	0	1.9	0
Total.....	-----	-----	-----	-----	501	588	43.5	11.1	3.9
Total calculated on 1:1 ratio.....	-----	-----	-----	-----	544	544	-----	-----	-----

F₃ GENERATION

Heavy losses of seed plants during the winter months of 1925-26 and 1926-27 from causes other than yellows delayed the study of the F₃ generation of these crosses. In the fall of 1927 about 100 F₂ plants were selected at random from plantings upon noninfested soil and removed to the greenhouse. About 30 of these produced seed. As a whole the seed plants were quite vegetative and many of them set little or no seed upon selfing. In order to test the genetic constitution of such plants and to provide a larger population for F₃ trials, back

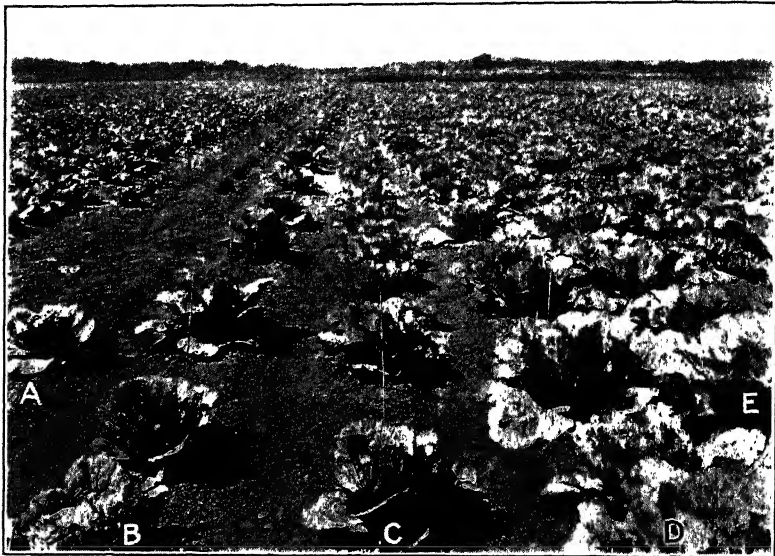


FIGURE 2.—Comparison of susceptible progenies, F₁ hybrids from a susceptible resistant cross, 2 F₂ progenies, and a back cross of an F₁ hybrid plant with a susceptible plant. Trials on naturally infested soil in Kenosha County, Wis., in 1927. A, Susceptible progenies, C-7×6, C-7s, and C-6s (Table 4); all plants except 1 were diseased. B, Back cross of an F₁ hybrid plant, G51-14, with a susceptible plant, C-12 (Table 11); 40 plants were diseased and 37, healthy. C, F₂ progeny, G51-21s (Table 8); 11 plants were diseased, 46 were healthy. D, F₂ selfed progeny of the F₁ plant used in the back cross shown in B, G51-14s (Table 8); 21 were diseased and 27, healthy. This was not a close fit to the expected 3:1 ratio. However, in the 1926 trial on the same field this progeny segregated into 10 diseased and 37 healthy. E, F₁ progeny from a susceptible-resistant cross, H8×5-28; all plants were healthy.

crosses were made in many instances, using pollen from plants known to be homozygous for susceptibility.

The seed of these plants did not mature in time to permit field tests to be carried out in the summer of 1928. Since in the meantime studies on the expression of resistance under controlled environment had shown that reliable tests for susceptible and resistant plants could be made in the greenhouse, these F₃ progeny tests were carried out in soil-temperature tanks. Soil used in several previous experiments and known to be thoroughly infested was used. The seedlings were started in noninfested soil and transplanted to the infested soil about three weeks later. After the plants had recovered from transplanting the soil temperature was raised to 23°-24° C. and held there.

The data collected from these trials (Table 12) include the results from selfed progenies and back crosses. In some cases the behavior of

only the back crosses was available to indicate the constitution of a given plant. In some cases also the population was smaller than might have been desired, but where the segregation was significant they were included in the totals. In three cases (5HII-2, G5II-7, and G35II-1) the parent plant was classed as homozygous susceptible, although one or two plants survived out of rather large populations in the selfed progenies. No satisfactory explanation can be offered for these exceptions. It is not believed that they escaped infection, since the soil used was thoroughly inoculated and the environmental conditions were ideal.

The four plants in these three progenies were undoubtedly resistant and obviously should not be present if they were true selfs from homozygous susceptible parents. For the present it may only be assumed that these were the result of pollen contamination.

Aside from the exceptions just noted and the back cross of H5II-5, the distribution of homozygous and heterozygous plants is about what might be expected from a monohybrid. It would be desirable, of course, to have a much larger number of F_2 plants tested, and this indeed would have been the case had not the unexpected complications already explained arisen.

TABLE 12.—Occurrence of yellows in F_2 cabbage progenies and in progenies of F_2 plants crossed with susceptible plants

[Tests made in the greenhouse upon thoroughly infested soil in soil-temperature-control tanks held at 23°–24° C.]

Original cross	F_2 plant No.	Selfed progenies		Back crosses with susceptible plants		Genotype class ^a
		Healthy plants	Diseased plants	Healthy plants	Diseased plants	
		Number	Number	Number	Number	
H-1×5-24	H5II-1			0	4	rr
	H5II-2	0	73			rr
	H5II-3	47	14			Rr
	H5II-4			105	0	RR
	H5II-5			14	2	Rr
H-1×5-25	H5II-14			2	4	Rr
	H5II-17			0	10	rr
	H5II-22			6	5	Rr
	5HII-1	11	0			RR
	5HII-2	1	98			rr
5-24×H-1	5HII-3			3	3	Rr
	5HII-4	3	0	8	7	Rr
	5HII-5			0	8	rr
	5HII-6	0	6			rr
	5HII-7	27	0	62	0	RR
	5HII-8	10	0			RR
	5HII-9	55	14	50	55	Rr
	5HII-10	61	17			Rr
	5HII-11	10	15			Rr
	5HII-13			77	0	RR
G-15×5-24	5HII-21			6	8	Rr
	G5II-1			10	0	RR
	G5II-3	6	1	39	42	Rr
	G5II-4	27	6			Rr
	G5II-7	2	76	0	15	rr
	G5II-8			15	0	RR
	G5II-9	87	22			Rr
	G5II-12			20	22	Rr
	G35II-1	1	23	0	15	rr
G-7×35-12	G35II-2	20	7	12	7	Rr
	G35II-3	7	0	28	30	Rr
	G35II-6			13	12	Rr

^a The actual number in each genotype class was RR (resistant), 7; Rr (heterozygous), 17; rr (susceptible), 8; and the expected numbers were 8, 16, and 8, respectively.

DISCUSSION OF CROSSES BETWEEN ALL HEAD EARLY AND GLORY OF ENKHUIZEN

When the behavior of the parent lines involved in these crosses of the F_1 hybrid and of the F_2 and F_3 families are considered, there seems little doubt that in these cases at least resistance is controlled by a single dominant Mendelian factor. It would be presumptuous to conclude, however, that this is generally the case in cabbage or its allies susceptible to this disease. One should also bear in mind the possibility of variation in the pathogene and the influence of environment upon the expression of resistance by the host. Attention has been called in the course of this report to variation in the response of plants in the susceptible class. How much of this variation is due to environment, and how much is due to heredity? May there enter in modifying factors which though failing to preclude infection and appearance of symptoms do retard progress of the disease? These and other questions are still open and worthy of further study. During the remainder of this paper evidence accumulated in other varieties along the line of that already presented will be discussed. The full discussion of the relation of environment to the expression of resistance is reserved for another paper.¹²

IN JERSEY WAKEFIELD VARIETY

In the fall of 1925 a few surviving healthy plants remained in the infested trial plots from a row of the commercial Jersey Wakefield variety. About 20 of these were planted in the greenhouse and 13 produced seed. Selfed progenies were secured from each, and four sib crosses were made. These were tested on the trial plot during 1926 and 1927. The data are given in Table 13. In all the selfed progenies and in all the sib crosses segregation was reasonably close to the ratio of 3 resistant to 1 susceptible. This indicated that, as already shown in All Head Early and Glory of Enkhuizen varieties, the few individuals that survived on infested soil were heterozygous for resistance. It also indicated that resistance is inherited in this variety in the same manner as in the other two studied.

TABLE 13.—Occurrence of yellows in first selections from the Jersey Wakefield variety of cabbage when grown upon infested soil

Progeny No.	Number of plants		Deviation	Probable error	Dev. P. E.
	Diseased	Healthy			
20-3s.....	4	21	2.3	1.5	1.5
20-6s.....	32	63	8.3	2.9	2.9
20-7s.....	24	69	.8	2.8	.3
20-8s.....	10	22	2.0	1.7	1.2
20-9s.....	20	48	3.0	2.4	1.3
20-10s.....	49	228	20.3	4.9	4.1
20-12s.....	33	111	3.0	3.5	.9
20-15s.....	9	68	10.3	2.6	4.0
20-17s.....	2	19	3.3	1.3	2.5
20-19s.....	5	12	.8	1.2	.7
20-21s.....	69	194	3.3	4.7	.7
20-29s.....	12	49	3.3	2.3	1.4
20-34s.....	23	62	1.8	2.7	.7
20-6×3.....	7	29	2.0	1.8	1.1
20-7×10.....	58	130	11.0	4.0	2.8
20-11×12.....	17	33	4.5	2.1	2.1
20-21×15.....	33	97	.5	3.3	.2
Total.....	407	1,255	8.5	11.9	.7
Total calculated on 1:3 ratio.....	415	1,247			

¹² WALKER, J. C., and SMITH, R. Op. cit.

Additional evidence on this point was secured in subsequent generations. Further selections were made from the surviving plants of the following progenies: 20-7s, 20-10s, 20-15s, 20-21s, 20-7×10, and 20-21×15. Seventeen plants in all were carried through, and the F_2 progenies tested. Where selfed progenies were not secured, the genetic constitution of the plants was tested by the behavior of back crosses with susceptible plants. Since in the parent progenies the susceptible plants, approximating 25 per cent of the total population in number, were eliminated by disease, it would be expected that of the remaining healthy plants approximately one-third would be homozygous for resistance and the remainder heterozygous. The data secured from the field tests of the progenies from selfed plants and those from back-crossed plants are given in Table 14. Six plants were homozygous for resistance, as shown by the behavior on selfing and back crossing to susceptible individuals. Of the remaining 11 plants, those that were tested by selfing segregated close to the 3 : 1 ratio. In the 1927 trials the plants from back crosses were grown on a recent addition to the plot where infestation with the causal organism was not so complete, and this resulted in a somewhat lower percentage of disease than was expected. When plants of which seed was still available were tested in 1928, the segregation was close to a 1 : 1 ratio.

In 1927 one homozygous plant (20-124) was crossed with a susceptible plant of Copenhagen Market (C-15) the selfed progeny of which was 100 per cent susceptible. (Table 4.) This hybrid progeny showed 1 slightly diseased plant out of 38. One of the F_1 plants of this cross (C-15×20-24) was selfed and the F_2 tested in 1928. Of a total of 43 plants, 12 were diseased and 31 healthy (theoretical, 10.7 and 32.3).

TABLE 14.—Occurrence of yellows in second-generation selections from the Jersey Wakefield variety of cabbage when selfed, and when crossed with susceptible plants; tests made upon infested soil

Class	Plant No.	Selfed progenies (1927)		Hybrid progenies			
		Diseased plants	Healthy plants	1927		1928	
				Diseased plants	Healthy plants	Diseased plants	Healthy plants
		Number	Number	Number	Number	Number	Number
Homozygous (RR) ^a	20-116	0	30				
	20-117	0	34	0	27		
	20-124	0	20	1	37		
	20-135	0	7	0	21		
	20-140	0	44	0	27		
	20-154	0	32	0	40		
	Total	0	167	1	152		
Heterozygous (Rr) ^a	20-115	8	22	17	27	24	17
	20-137	6	20	4	9	33	22
	20-138	6	7				
	20-155	11	21	8	49		
	20-157	17	42	15	34		
	20-158	9	27	9	34		
	20-109			16	34	27	35
	20-131			9	18	20	28
	20-136			12	14	15	13
	20-159			11	7		
	20-139			3	19	18	18
	Total	57	139	104	245	137	133

^a Theoretical number, 5.6.

^b Recovered.

^c Theoretical number, 11.3.

Thus it is clear that in the Jersey Wakefield variety resistance is inherited as a single dominant factor in the same manner as in All Head Early and Glory of Enkhuizen.

IN COPENHAGEN MARKET VARIETY

In 1925 a number of resistant individuals were selected from a planting of Copenhagen Market variety on infested soil. As in the case of Jersey Wakefield, selfs and sib crosses were made during the following winter, and progeny tests were made in 1926 and 1927. The data given in Table 15 show that in all cases but two (21-27s and 21-49s), where the population was small, segregation was close to the ratio of 3 resistant to 1 susceptible. Of the surviving plants in these trials, 8 were grown to seed and their genetic constitution tested by back crossing with susceptible plants. Since the homozygous susceptible plants presumably had been eliminated by disease, approximately one-third of these should have been homozygous resistant and two-thirds heterozygous. This test showed 3 to be homozygous resistant and 5 to be heterozygous. The inheritance of resistance in this variety of cabbage is, therefore, similar to that of other varieties studied.

TABLE 15.—Occurrence of yellows in first selections of resistant individuals from Copenhagen Market (commercial variety of cabbage) showing 3:1 segregation when grown upon infested soil

Progeny No.	Number of plants		Deviation	Probable error	Dev. P. E.
	Diseased	Healthy			
21-27s.....	10	11	4.8	1.3	3.7
21-28s.....	50	165	3.8	4.3	.9
21-31s.....	12	45	2.3	2.2	1.0
21-32s.....	2	11	1.3	1.1	1.2
21-33s.....	2	5	.3	.8	.4
21-44s.....	4	19	1.8	1.4	1.3
21-49s.....	14	15	6.8	1.6	4.3
21-53s.....	4	13	.3	1.2	.3
21-56s.....	11	21	3.0	1.7	1.8
21-60s.....	6	22	1.0	1.6	.6
21-29×30.....	8	19	1.3	1.5	.9
21-30×28.....	10	36	1.5	2.0	.8
21-30×29.....	13	36	.8	2.0	.4
21-41×44.....	6	13	1.3	1.3	1.0
21-44×41.....	5	13	.5	1.2	.4
Total.....	157	444	6.8	7.2	.9
Calculated.....	150	451			

IN WILD CABBAGE

As was stated in a previous article,¹³ *Fusarium conglutinans* is apparently confined in its parasitic relations to forms of *Brassica oleracea*. The wild form of this species is native to certain parts of Europe where yellows does not occur. A small sample of seed of wild cabbage was secured from England. Of 17 plants grown on infested soil, 1 succumbed to yellows. From the remaining healthy plants, 3 were saved and selfed seed were secured from each of them. These progenies were tested on the trial grounds in 1926, 1927, and 1928. It will be seen from the data given in Table 16 that each of these segregated as monohybrids. This shows that in the wild subspecies resistance is inherited in the same manner as in certain cultivated varieties of cabbage.

¹³ WALKER, J. C., and WELLMAN, F. L. A SURVEY OF THE RESISTANCE OF SUBSPECIES OF *BRASSICA OLERACEA* TO YELLOW (FUSARIUM CONGLUTINANS). Jour. Agr. Research 37: 233-241, illus. 1928.

TABLE 16.—Occurrence of yellows in selfed progenies of wild cabbage plants when grown upon infested soil

Progeny No.	Year tested	Number of plants		Deviation	Probable error	Dev. P. E.
		Diseased	Healthy			
WC-1-----	1926	6	17	0.3	1.4	0.2
	1927	7	13	2.0	1.3	1.5
	1928	12	37	.3	2.0	.2
Total-----		25	67	2.0	2.8	.7
WC-2s-----	1926	1	7	1.0	.8	1.3
	1927	6	12	1.5	1.2	1.3
Total-----		7	19	.5	1.5	.3
WC-3-----	1926	4	21	2.3	1.5	1.5
	1927	9	51	6.0	2.3	2.6
	1928	10	21	2.3	1.6	1.4
Total-----		23	93	6.0	3.2	1.9
Grand total-----		55	179	3.5	4.5	.8
Grand total calculated on 1:3 ratio-----		58	176			

DISCUSSION

The main purpose of this study was to secure more exact information about the heredity of resistance to the attack of *Fusarium conglomeratum* in cultivated forms of cabbage. The evidence as a whole leads to the conclusion that in the varieties studied the majority of the plants are homozygous for susceptibility. There are practically always a few plants that resist the parasite under very favorable conditions for the development of disease. Such individuals are usually heterozygous, and when they are selfed, and the next generation tested, there is a segregation of approximately 25 per cent susceptible to 75 per cent resistant individuals. Further selection from the resistant plants yields progenies that are completely resistant under the natural field conditions employed.

Starting with homozygous susceptible and homozygous resistant individuals as parents, the F_1 hybrids show resistance equal to the resistant parent. When such hybrids are carried to the F_2 generation, segregation is that characteristic of a monohybrid, the actual counts showing segregation of approximately 3 resistant to 1 susceptible. When the F_2 generation was grown on noninfested soil to avoid elimination of susceptible individuals and plants were tested as to their genetic constitution by growing of F_3 progenies or of back crosses to susceptible plants, they were shown to consist of approximately one-fourth homozygous susceptible, one-half heterozygous, and one-fourth homozygous resistant.

In the plants studied, which included four varieties of cultivated cabbage and the wild cabbage, all evidence points to the conclusion that resistance is governed by a single dominant Mendelian factor. The extension of this study to the other cultivated subspecies of *Brassica oleracea*, such as cauliflower, kale, kohlrabi, and Brussels sprouts, would be of interest. All varieties of these so far tested show segregation into classes resistant and susceptible to the yellows organism.¹⁴

¹⁴ WALKER, J. C., and WELLMAN, F. L. Op. cit

While the evidence so far secured has been consistent with an assumption of single-factor inheritance, it is not implied that this is necessarily true throughout the host range of this parasite. In fact, it does not preclude the possibility of other types of resistance than the one herein described, nor is the writer able, at the present time, to explain with complete satisfaction the variation that was consistently observed in the rate at which the disease developed in the so-called susceptible populations. In the case of a disease which is so profoundly affected by environment, one would expect to find an explanation of part of this variation in environmental rather than in genetic factors. An extensive study of a number of factors influencing the expression of resistance and susceptibility has been made,¹⁵ and it is quite evident from the results thereof that a part of the variation referred to is due to environment and to the age of the plants when exposed to the parasite, but it is equally clear that all of the observed differences can not be so explained. In fact, as more observations are made and more data accumulate it appears increasingly likely that intermediate forms may be isolated. It is reasonable to suppose that hereditary factors acting quite independently of the main gene determining resistance may modify the expression of the disease. This is indicated by the results obtained with a comparatively few susceptible progenies recently acquired; though these show practically 100 per cent diseased plants under field conditions, a majority of them are only mildly affected, while other progenies in adjacent rows succumb promptly and completely. The first-named type might be designated as "mildly susceptible under field conditions." Its lack of true resistance is shown, however, when exposed to an environment somewhat more favorable to the parasite (constant soil temperature of 23°–24° C.), where the young plants succumb completely, while those in progenies designated as homozygous for resistance remain perfectly healthy.

The importance of more study of these finer differences is paramount from a practical standpoint. In the development of resistant varieties the lack of very close scrutiny may easily lead to the selection and perpetuation of mildly susceptible types, particularly where mass selection is practiced and where variations in soil infestation by the parasite and in seasonal climatic conditions preclude uniformly severe testing for resistance. Thus so-called resistant varieties may contain considerable percentages of such mildly susceptible individuals which remain healthy in certain seasons and in certain localities, but become diseased when exposed to an environment slightly more severe. It is strongly suspected that the mass-selected resistant varieties of cabbage, such as Wisconsin Hollander, do contain an appreciable percentage of individuals of this sort. In the light of the present findings it should be possible to improve such varieties by self-pollination and the elimination of "mildly susceptible" plants.

The possible variation in the pathogenicity of the parasite has not been overlooked. Since this is being made the subject of a special study by L. M. Blank, it is not necessary to discuss it in detail here, except to note that *Fusarium conglutinans* is quite constant in its pathogenicity so far as the study of numerous strains from widely separated localities indicates.

¹⁵ WALKER, J. C., and SMITH, R. Op. cit.

In this study no evidence has been acquired that resistance is linked with any of the important type characters, such as color of foliage, shape of head, length of stem, vigor of plants, and time of maturity. It therefore seems reasonable to expect that no serious difficulty will be encountered in securing yellows-resistant strains that conform closely to the standard varieties already adapted to the needs of various markets.

SUMMARY

This paper reports a study of the nature of inheritance of resistance to the yellows organism (*Fusarium conglutinans*) in a number of cabbage varieties and in wild cabbage.

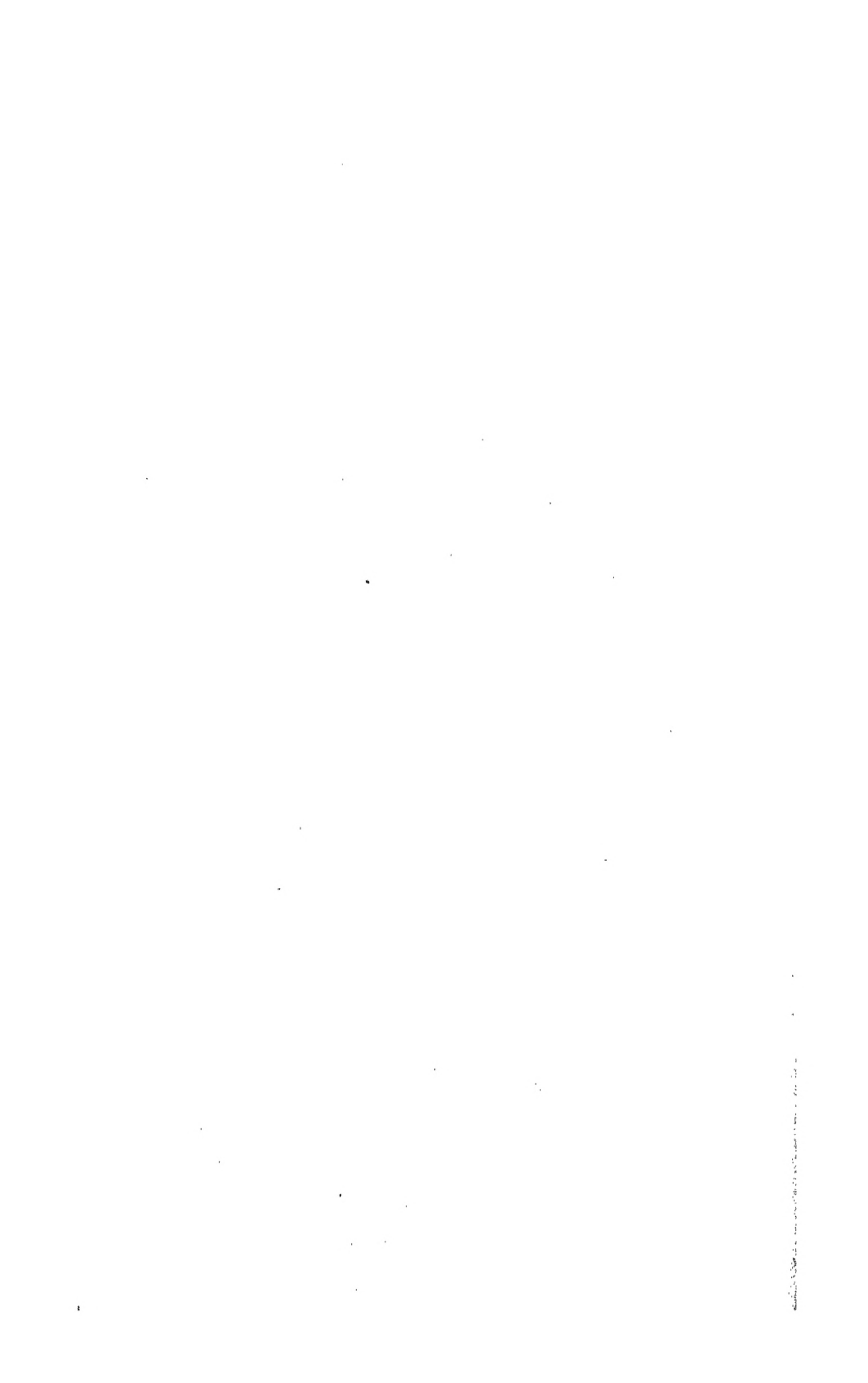
The symptoms of the disease and the method of testing progenies in the field for resistance and susceptibility are described.

Homozygous resistant and homozygous susceptible lines were selected first from All Head Early and Glory of Enkhuizen varieties. F_1 hybrid progenies from crosses between resistant and susceptible individuals showed resistance equal to the resistant parental lines.

In the F_2 progeny tests the segregation was, in practically all cases, a very close fit to the ratio of 3 resistant to 1 susceptible expected from a monohybrid. Back crosses between F_1 hybrid plants and susceptible plants yielded progenies which segregated close to the expected 1 to 1 ratio. A group of F_2 plants grown on noninfested soil and studied as to their genetic constitution by F_3 progeny tests, and by back crossing to susceptible plants, was shown to consist of approximately 25 per cent homozygous resistant, 50 per cent heterozygous, and 25 per cent homozygous susceptible plants.

Studies of the Jersey Wakefield variety, the Copenhagen Market variety, and wild cabbage show that in these three cases resistance to yellows is also controlled by a single gene.

The practical importance of recognizing and eliminating mildly susceptible strains from mass-selected resistant varieties is emphasized. No data have so far tended to show that resistance to yellows is linked with any important type character.



EFFECT OF THE APPLE STRAIN OF THE CROWN-GALL ORGANISM ON ROOT PRODUCTION¹

By E. A. SIEGLER

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INTRODUCTION

The excessive development of roots frequently found on malformations of the apple and variously termed "woolly knot," "crown



FIGURE 1.—A root cutting with some of the shoots removed. Inoculations were made at the base of these detached shoots. Natural size

gall," or "hairy root" has been noted by numerous investigators. In commenting on this, Riker et al.² reported the isolation of organisms resembling *Bacterium tumefaciens* Sm. and Town. from "naturally occurring malformations" on apple roots. These investigators made inoculations either on the underground parts of young apple trees or on the underground parts of shoots springing from the scion. They

¹ Received for publication Jan. 14, 1930; issued April, 1930.

² RIKER, A. J., BANFIELD, W. M., WRIGHT, W. H., and KEITT, G. W. THE RELATION OF CERTAIN BACTERIA TO THE DEVELOPMENT OF ROOTS. *Science* (n. s.) 68: 357-359. 1928.

concluded that the organism they used was different from the crown-gall organism in certain respects, and that these differences were such as probably to be of specific significance. Emphasis was placed on the fact that the organism used by them was "root-stimulating," and the question was raised as to the possible utilization of this organism in commercial practice.

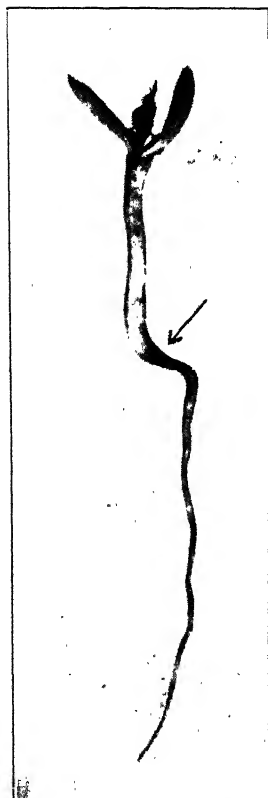


FIGURE 2.—Seedling cut at point designated by arrow and inoculated at basal end. Natural size

Siegler³ had reported the presence of the apple strain of the crown-gall organism in malformations on apple roots. The assumption that the organism he used was identical with the apple strain of Smith et al.⁴ was based on the fact that he obtained results similar to those obtained by these workers. Riker et al.⁵ did not comment on the possibility that their organism might be identical with one used by Smith and his coworkers and by Siegler, but from their report it seems probable that the so-called apple strain is identical with the organism designated by Riker et al. as "root-stimulating."

Siegler and Piper⁶ concluded that the apple strain stimulated the production of root primordia in the stem tissue of the apple. Attention was called to the fact, however, that after their initiation these root primordia could be pushed into active growth, in the apparent absence of bacteria, by planting in the soil. In this way malformations known as "woolly knots" were produced. The experiments reported here are thought to furnish evidence bearing directly on the question whether or not these organisms, per se, can be considered as "root stimulating."

EXPERIMENTS

The experiments were planned to furnish information on the effect of this organism on certain tissues of the apple. Shaw⁷ showed the practicability of growing apple trees from root cuttings. This suggested to the writer the idea of using the shoots from such cuttings for inoculation purposes. The roots used were seedlings of the Rasmussen variety. The root cuttings, approximately 3 inches in length from 1-year-old growth, were placed in sand and kept under such conditions as to force sprouting. When the sprouts were from 1 to 3 inches long they were excised from the root cutting, as shown

³ SIEGLER, E. A. STUDIES ON THE ETIOLOGY OF APPLE CROWN GALL. *Jour. Agr. Research* 37: 301-313, illus. 1928.

⁴ SMITH, E. F., BROWN, N. A., and TOWNSEND, C. O. CROWN GALL OF PLANTS: ITS CAUSE AND REMEDY. U. S. Dept. Agr., Bur. Plant Indus. Bul. 213, 215 p., illus. 1911.

⁵ RIKER, A. J., BANFIELD, W. M., WRIGHT, W. H., and KEITT, G. W. Op. cit.

⁶ SIEGLER, E. A. and PIPER, R. B. AERIAL CROWN GALL OF THE APPLE. *Jour. Agr. Research* 39: 249-262, illus. 1929.

⁷ SHAW, J. K. THE PROPAGATION OF APPLE TREES ON THEIR OWN ROOTS. *Mass. Agr. Expt. Sta. Bul.* 190, p. [73]-96, illus. 1919.

in Figure 1. Inoculations were made with the apple strain and with other strains of the crown-gall organism by smearing the base of these sprouts on agar cultures. They were then planted in sand in 6-inch pots. In addition to the sprouts from the root cuttings, young shoots growing from seed were used. These were cut off, approximately one-eighth inch below the junction of the root and shoot tissue (fig. 2), and the bases, including the one-eighth inch of root tissue, were inoculated and planted in the same manner as the sprouts obtained from the root cuttings. The controls received no inoculations, but it should be noted (Table 1) that certain other strains of the crown-gall organism, such as Nos. 491 and 637, also served as controls for the apple strain. Strains 491 and 637 cause the smooth type of malformation which the writer⁸ has previously illustrated and which is in sharp contrast to the woolly-knot type of malformation caused by the apple strain. In previous experiments, inoculations had been made with the apple strain on 3-inch to 5-inch apple seedlings at the



FIGURE 3.—The lot of apple seedlings in experiment 270, showing root inhibition as a result of inoculation with the apple strain. $\times \frac{1}{2}$

ground line, with results in agreement with those reported by Riker et al.,⁹ in that an apparent root stimulation is produced when inoculations are made in this region by punctures.

DISCUSSION OF RESULTS

Reference to Table 1 shows that the apple strain of the crown-gall organism which produces the woolly-knot type of crown gall¹⁰ with its usual production of large amounts of roots failed to stimulate root development on these shoots which might be considered as soft-wood cuttings of the apple. On the contrary, in all experiments except one (No. 296), the action was decidedly a root-inhibiting one. The two lots of shoots which were dug in experiment 270 are shown in Figures 3 and 4, which illustrate the apparent root-inhibiting effect when inoculations are made with the apple strain (fig. 3) as compared with the uninoculated control lot (fig. 4). The injurious effect of the organism on the growth of the seedlings is also shown in this comparison. That this organism did not form merely a mechanical barrier

⁸ SIEGLER, E. A. THE WOOLLY-KNOT TYPE OF CROWN GALL. Jour. Agr. Research 39: 427-450, illus. 1929. (Fig. 6.)

⁹ RIKER, J. A., BANFIELD, W. M., WRIGHT, W. H., and KEITT, G. W. Op. cit.

¹⁰ SIEGLER, E. A. Op. cit. (See footnotes 3 and 8.)

TABLE 1.—Results obtained by inoculating apple cuttings and seedlings with the apple strain and other strains of the crown-gall organism

Experiment No.	Inoculum	Origin of shoots	Date inoculated and planted	Date dug	Number of shoots		Results			Percent- age of surviving shoots showing root develop- ment
					Planted	Surviv- ing	Number of shoots of shoots showing no root develop- ment		Thick	
							Sparse	Thick		
227-A	Apple strain 486-107	Root cutting	1929	1929	15	8	0	0	8	0
227-B	491-108 control	do	Mar. 9	Apr. 18	13	9	2	4	4	66
228-A	Apple strain 486-107	do	do	do	15	10	0	0	10	0
228-B	Uninoculated control	do	do	do	12	9	3	5	1	89
269-A	Apple strain 486-107	do	May 10	June 10	15	11	6	2	3	73
269-B	Uninoculated control	do	do	do	15	13	0	13	0	100
270-A	Apple strain 486-107	do	do	do	10	6	2	0	4	33
270-B	Uninoculated control	do	do	do	10	7	1	5	1	86
298-A	Apple strain 486-107	Seed	May 23	July 2	10	7	0	7	0	100
298-B	Uninoculated control	do	do	do	10	7	0	7	0	100
302-A	Apple strain 486-107	do	June 18	July 11	10	6	1	1	4	33
302-B	Uninoculated control	do	do	do	10	10	3	4	0	100
308-C	491-108 control	do	do	do	10	10	0	9	1	90
310-A	Apple strain 486-107	do	June 20	do	10	7	1	0	6	14
310-B	Uninoculated control	do	do	do	10	8	1	5	2	75
310-C	Peach strain control	do	do	do	10	9	1	8	0	100
310-D	637-109 control	do	do	do	10	7	2	5	0	100
Total apple strain 486-107					85	55	10	10	35	86
Total uninoculated controls					70	50	8	35	7	86
Total 491-108 controls					23	19	2	13	4	73
Total peach strain controls					10	9	2	3	0	100
Total 637-109 control					10	7		5	0	100

to root production is indicated by results obtained when other strains of the crown-gall organism were used. In these cases root development proceeded the same as on the uninoculated controls. Of the 55 shoots surviving in the lots inoculated with the apple strain, only 36 per cent showed root development, whereas of the 50 shoots surviving in the uninoculated lots, 86 per cent showed root development.

In contrast to these results, there appears to be a root stimulation following inoculations with this organism in regions other than those described. Figure 5 shows the excessive root development usually obtained as a result of inoculations on 3-inch to 5-inch seedlings at a point near the collar. Thus the nature of the results obtained depends upon the region inoculated, and it is questionable which of these two regions should be considered the criterion of the "root-stimulating" or "root-inhibiting" powers of this organism. In general, the action of organisms or other factors on cuttings of other

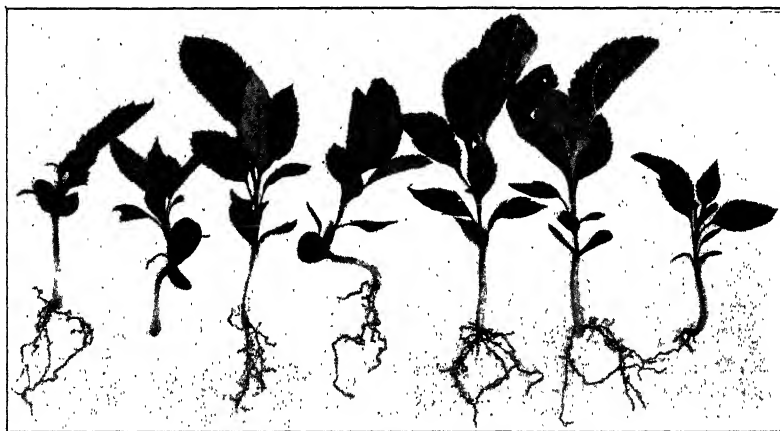


FIGURE 4.—The uninoculated control lot of apple seedlings in experiment 270, showing normal root development. $\times \frac{1}{2}$

hosts would probably be the standard by which they would be judged. In these experiments the sprouts from the root cuttings correspond most closely to what are termed "soft-wood cuttings" in commercial practice. Viewed from this standpoint, the organism actually inhibits root development.

It is difficult to explain why an organism will cause apparent root stimulation when introduced into one region of a root and what apparently is an inhibiting action when introduced into another region. The two regions at which the inoculations were made, however, can hardly be considered analogous, especially from the standpoint of elaborated food and food materials. There is probably a greater concentration of elaborated food at the base of the excised shoot than there is farther up the stem. The writer has advanced the opinion that the food supply may be an important factor in connection with the occurrence of the woolly-knot type of crown gall. In fact, it was to test this hypothesis that the idea was conceived of performing the experiments described here, whereby the food supply received by the bacteria from the plant would consist of elaborated food only, without the usual accompaniment of transported food materials. There are

numerous other factors which undoubtedly influence the interaction of the organism and the host at the points of inoculation and it should be recalled that woolly knots, practically free of root developments, are frequently encountered.

The frequently observed phenomenon that girdling results in the production of roots above the girdled area prompts the explanation that the bacteria may, in some direct or indirect way, cause a girdling action when inoculated at a point some distance up from the base of the shoot. Because of its plausibility, this explanation is offered as to why root production results when inoculations are made part way



FIGURE 5.—Root production following inoculation with the apple strain at the collar of an apple seedling. Note paucity of roots in other regions. Natural size.

up the shoot. Moreover, the seedling shown in Figure 5 is held to show "root stimulation" more apparent than real, because the excessive amount of roots at the point of inoculation is counterbalanced by the resulting paucity of roots in the remaining regions. The illustration represents what might readily have happened if mechanical girdling had taken place in this region. The region below the part indicated by the arrow can not function normally. This same condition is frequently observed on nursery trees exhibiting large "woolly knots" at the graft union, and in older trees where a mass of fine roots at the collar constitutes practically the entire root system, allowing the tree to be easily uprooted. Naturally occurring woolly-knot

types of galls, when found on the basal end of the seedling part of an apple tree, almost invariably are void of root developments, whereas woolly knots at the graft union usually exhibit profuse root developments. Thus there is close agreement between the results of these experiments and the phenomena observed in nature.

SUMMARY

Inoculations with an organism considered to be identical with the apple strain of *Bacterium tumefaciens* Sm. and Town. are reported.

When inoculations were made at the cut ends of apple shoots grown from root cuttings and at the base of apple shoots grown from seed, from which most of the root systems were removed, root production was inhibited and growth was retarded.

Since the base of the shoots obtained from root cuttings and from seeds is comparable to the base of soft-wood cuttings, the organism might be considered as inhibiting root production; but since excessive root development frequently results from inoculations at other regions, it is questionable if this organism, per se, possesses either direct root-stimulating or root-inhibiting powers.

INFLUENCE OF BORDEAUX MIXTURE ON THE EFFICIENCY OF LUBRICATING-OIL EMULSIONS IN THE CONTROL OF THE SAN JOSE SCALE¹

By B. A. PORTER, *Entomologist*, and R. F. SAZAMA, *Assistant Entomologist, Division of Deciduous-Fruit Insects, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

Oil sprays are sometimes combined with fungicides, particularly with those containing copper, for the simultaneous control of insects and plant diseases. The question arises whether in such combinations the oil retains its full insecticidal efficiency. Experimental data that will throw light on this question have been accumulated at the field station maintained at Vincennes, Ind., by the Bureau of Entomology in cooperation with the Purdue University Agricultural Experiment Station.

The work here reported is of interest chiefly in connection with the dormant spraying of peach for the combined control of peach leaf curl and the San Jose scale (*Aspidiotus perniciosus* Comst.), both of which must be controlled in the dormant period. For a long time prior to 1921 this control was accomplished by the use of lime-sulphur, which ordinarily gives satisfactory control of the scale and has been for years the standard remedy for leaf curl. With the widespread adoption of oil emulsions, chiefly those made according to the formula developed by Yothers (8)² and first found to be effective in the control of the San Jose scale by Ackerman (2), many peach growers began to use a combination of oil and Bordeaux mixture for dormant spraying; and, though there is some conflict of opinion as to the effectiveness of the Bordeaux mixture in the control of peach leaf curl when used in combination with oil, this spray is now in fairly common use. Some growers have substituted for the usual Bordeaux mixture a solution of copper sulphate, omitting the lime.

Another common use of Bordeaux mixture is in maintaining a stable mixture when hard water must be used for diluting the oil emulsion. The calcium or magnesium salts present render the soap insoluble, liberating the oil, which then rises to the surface in a continuous layer. Yothers (8, revised) found that this difficulty could be overcome by diluting the soap-oil emulsion with a weak Bordeaux ($\frac{1}{2}$ - $\frac{1}{2}$ -50 or $\frac{1}{4}$ - $\frac{1}{4}$ -50).³ This prevents the separation of the oil, although a distinct reaction does occur which will be discussed later.

Bordeaux mixture has also been found to be a good emulsifier (7, p. 2011), some growers preparing the spray in the tank of the spray rig and emulsifying the raw oil with Bordeaux mixture by the action of the pump and the agitator. Oil emulsion is also sometimes in

¹ Received for publication Dec. 4, 1929; issued April, 1930.

² Reference is made by number (italic) to "Literature cited," p. 765.

³ The usual practice is followed in referring to the various formulas for Bordeaux mixture. The first term gives the quantity of copper sulphate (in pounds), the second the quantity of hydrated lime (in pounds), and the third the total quantity of spray liquid (in gallons). For brevity, copper sulphate solution alone is referred to in the tables as 1-0-50 or 2-0-50 Bordeaux. The oil concentrations mentioned in this article refer to the percentage by volume of oil present in the completed spray mixture.

emergencies added to summer sprays of Bordeaux mixture in the attempt to obtain a partial control of the scale on apple.

Most of the published statements indicate that Bordeaux mixture does not lessen the insecticidal efficiency of an oil spray with which it is combined. An exception to this general opinion is a report by Harman (5) showing a decreased kill of cottony peach scale when Bordeaux mixture was added to the oil spray.

The comparative tests against the San Jose scale on which the published statements are based appear to have been made with oil concentrations high enough for all the mixtures to give practically perfect control. Differences between materials may be overlooked, however, unless the tests include dilutions below the point of full effectiveness.

REACTIONS

When a soap-oil emulsion is combined with Bordeaux mixture the soap appears to react at once with the calcium or copper present, forming an insoluble calcium or copper soap. Instead of being liberated, however, the oil remains intimately associated with the Bordeaux mixture, and the two together tend to form a definite layer separate from the water in the diluted spray mixture. This separation, or "layering," is of no consequence in ordinary spraying work, since present-day outfits provide sufficient agitation to maintain a uniform mixture as long as the oil alone has not formed a separate layer.

When copper sulphate alone is added to a soap-oil emulsion, a similar reaction occurs. The resulting insoluble copper soap remains in a finely divided condition, and seems to give a sufficient volume of solid material to prevent the immediate separation of the oil. The volume of copper soap is so small, however, that the mixture layers as described above very rapidly, and vigorous agitation is needed to maintain a uniform emulsion.

EXPERIMENTAL METHODS

The experiments here reported were conducted entirely in the orchard, under field conditions, since the San Jose scale does not lend itself readily to laboratory manipulation. As the spread of the scale from tree to tree is comparatively gradual and depends wholly on chance transportation by wind or other agencies, there was no necessity for treating large areas. In the dormant season, when no movement of scales could occur, the treated areas ranged from a single, large, well-infested branch to four entire trees. In summer tests, from two to four trees were used in each plot. The materials were applied with a power outfit, or with a large hand pump which gave a pressure of approximately 150 pounds and delivered a spray very similar to that obtained with a regulation power outfit.

Except in the earliest experiments, three or four concentrations of oil were included in the test of each combination. Some of the concentrations were well below the point where complete kill was to be expected, in order that a toxicity curve could be worked out for each material. A curve of this nature gives much more complete information about an insecticide than would the result of a single test, since it gives an indication of the approximate concentration at which full effectiveness is reached.

Beginning with the tests conducted during the winter of 1926-27, the actual oil content of each dilution was determined by the method suggested by Griffin and Richardson (4). The samples were taken directly from the nozzle, usually one sample at the start of the spraying and a second at the close. The agreement between the two samples was almost without exception satisfactory. This procedure has been found particularly valuable in field work with oil sprays, since a number of factors conspire to cause inaccuracies in mixing small quantities of spray material in a large power outfit. The irregularities revealed by these analyses render it difficult to make direct comparisons from the control data given in Tables 3 and 4, and much better comparisons may be made from the toxicity curves in Figures 3 and 4.

The differences in efficiency caused by the addition of Bordeaux mixture first appeared in tests conducted during the growing season, but summer work was soon dropped, as the use of oil in the summer for the control of the San Jose scale is resorted to only in emergency cases on apple and practically never on peach. Experiments in the control of the scale during the growing season are extremely unsatisfactory, on account of the irregularity of spray coverage caused by the interference of the foliage and the rapid changes which occur in the relative numbers of scales of different ages during the interval between spraying and the recording of results.

In scoring the tests of summer treatments, all the scales found were counted except the very small ones which had evidently appeared after the materials were applied. In the dormant tests, only the partially grown, hibernating forms were counted; the more nearly mature scales and those which are born just before the trees become dormant never survive the winter in southern Indiana and could therefore be disregarded. Material for counting was obtained at random from various portions of the branch, tree, or block treated, where the scale population was sufficiently great to permit satisfactory scoring. The counts were usually divided about evenly between old, roughened wood, where conditions approached incrustation, and the young growth where the scale was usually less abundant. Extremely heavy incrustations were not included, since an exact count under such conditions is very difficult, although it is realized that a somewhat higher concentration of oil is needed to penetrate the scale coverings when there is a great deal of overlapping, as has been shown by Bliss⁴ in the case of the camphor scale. These experiments, however, were an attempt to measure the influence of Bordeaux mixture on the effectiveness of the oil rather than to ascertain the exact dilutions needed in commercial spraying.

Summer tests were scored from 10 to 14 days after the sprays were applied. During the dormant season, however, the dead scales dry out very slowly, and it is sometimes as much as three months before they can be distinguished from the live ones with any degree of certainty.

In most of the experiments at least 1,000 scales were counted for each treatment, but in the spring of 1925 the normal mortality reached the unusually high mark of 79 per cent, so during that season the size of the counts was doubled, 2,000 scales being the standard. As the normal mortality varied considerably from year to year, that which

⁴BLISS, C. I. Unpublished manuscript.

could be attributed to the material applied has been calculated for all sprayed plots by the usual methods (1), and is referred to as "per cent efficiency." This permits the combining of the results from different sets of experiments on a fairly comparable basis.

OILS USED

Three different lubricating oils, the specifications for which are given in Table 1, were used in the tests reported here. All were of the general type used in preparing oil sprays. Oil No. 1 is the one in most common use for dormant spraying in the Middle West, and for that reason was used in most of the experimental work.

TABLE 1.—Specifications¹ for the lubricating oils used in the experimental sprayings, Vincennes, Ind., 1923 to 1928

Oil No.	Density at 20° C.	Volatility (4 hours at 105° C.)	Viscosity (Saybolt at 100° F.)	Unsulpho-nated residue	Base
1-----	0.880-0.887	<i>Per cent</i> 0.33-1.01	<i>Seconds</i> 96-108	<i>Per cent</i> 64.4-65.6	Paraffin or mixed.
2-----	.899	.010	229	54.4	Do.
3-----	.922	.88	310	59.2	Naphthalene.

¹ Analyses made by the Insecticide and Fungicide Board (now a part of the Food, Drug, and Insecticide Administration), U. S. Department of Agriculture.

EXPERIMENTAL DATA

SUMMER TESTS

Three experiments, all on apple, were conducted during the summer of 1923, in which oil No. 1 was used, and the materials applied with a power outfit.

The first tests were conducted on Grimes Golden during the early part of July, when the most advanced first-brood scales were about three-fourths grown. In this experiment, counts of equal numbers were made on both twigs and fruit, and the resulting figures combined. The oil was used at concentrations of 1 and 2 per cent, with two strengths of Bordeaux mixture— $\frac{1}{2}$ - $\frac{1}{2}$ -50 (used because of the hardness of the water) and 2-3-50. The second test was a repetition of the first, but made on Winesap trees late in July when the very earliest crawlers of the second generation were appearing. During the period when these two experiments were being conducted the temperatures were very high, reaching 95° F. or more nearly every day.

The third experiment was started in the middle of September, when the temperatures averaged much lower. At this time crawlers of late broods were appearing daily. In this test the counts were restricted to the twigs.

The data obtained in these three experiments are shown in graphic form in Figure 1.

During the summer of 1924 similar experiments were conducted on apple.⁵ Two oils were used, each with $\frac{1}{2}$ -1-50 and 3-5-50 Bordeaux and without Bordeaux mixture. The series with oil No. 1

⁵ The senior author was assisted during the summer of 1924 by B. E. Montgomery.

was repeated three times, and since these three tests were conducted within a very short period in the same orchard, and since the results of the individual tests were very similar, the three tests have been combined and reported as a single one.

The varieties in this orchard were mixed, and it was impossible to use the same variety for the entire series, but counts of scales from the different varieties revealed no significant differences in mortality. In most cases one Grimes Golden tree and one of another variety

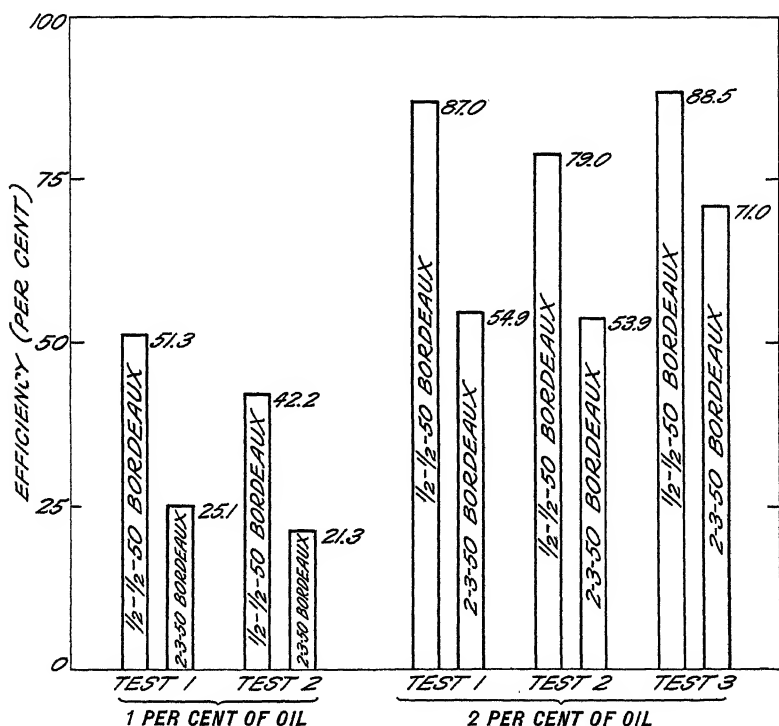


FIGURE 1.—Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the summer control of the San Jose scale on apple, Vincennes, Ind., 1923. Oil No. 1 was used throughout, emulsified with potash fish-oil soap by the boiling process. The counts include scales in all stages of development

were used for each portion of the test. The materials were applied with the hand outfit already described.

The results of these tests are summarized graphically in Figure 2.

TESTS DURING THE DORMANT SEASON

Previous to 1925 all tests made at Vincennes during the dormant period included only concentrations of oil so high that all gave practically complete control. In the spring of 1925, however, concentrations as low as 1 per cent of oil were included in the tests. Oil No. 1 was used without Bordeaux mixture, with a 1/2-1-50 Bordeaux, and with a 4-6-50 Bordeaux. The materials were applied to peach trees with a power outfit, and from one to four trees were included in each plot.

The data obtained are shown in Table 2, and these in part have also been combined with results of later experiments with the same oil in plotting the curves shown in Figure 4.

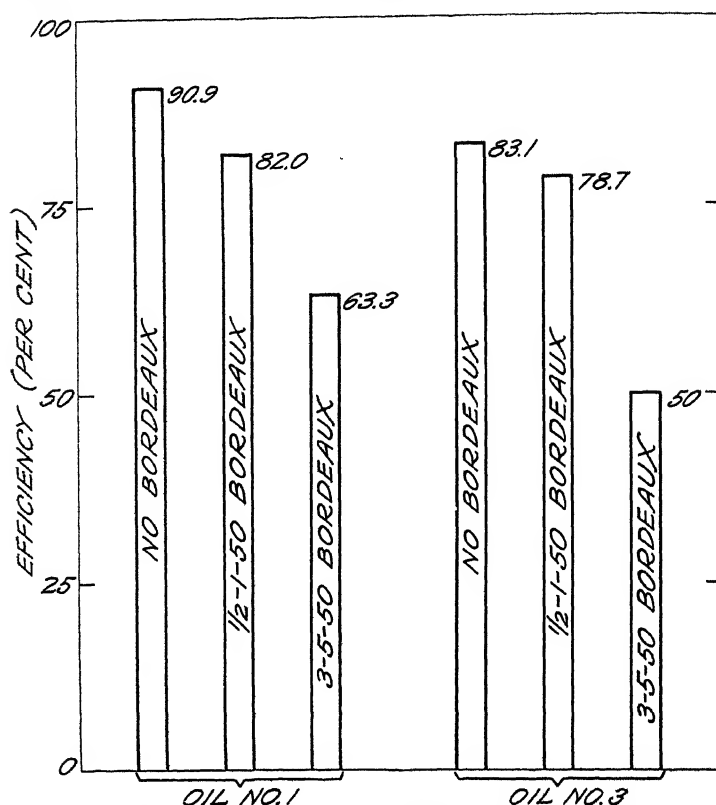


FIGURE 2.—Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the summer control of the San Jose scale on apple, Vincennes, Ind., 1921. The oils were emulsified with potash fish-oil soap by the boiling process, and used at 2 per cent in all the tests. The counts include scales in all stages of development.

TABLE 2.—Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., spring of 1925

[Oil No. 1, emulsified with potash fish-oil soap, by the boiling process, was used throughout]

Plot No.	Strength of Bordeaux mixture	Strength of oil used	Scales counted	Scales dead	Efficiency of spray
		Per cent	Number	Per cent	Per cent
1	None used	1.0	2, 018	99.9	99.5
2		1.5	2, 014	99.4	97.1
3		2.0	2, 045	100.0	100.0
4		3.0	2, 062	100.0	100.0
5	1/2-1-50	1.0	2, 026	95.9	80.3
6		1.5	2, 045	97.9	89.9
7		2.0	2, 030	99.9	99.5
8		3.0	2, 013	99.7	98.6
9	4-6-50	1.0	2, 022	93.6	69.2
10		1.5	2, 048	99.1	95.7
11		2.0	2, 036	99.8	99.0
12		3.0	2, 084	100.0	100.0
13	Check (no treatment)		2, 030	79.2	

TABLE 2.—*Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., spring of 1925—Continued*SIGNIFICANCE OF DOUBTFUL DIFFERENCES¹

Plots compared	Arithmetic difference	P. (probability that the difference was accidental)
1 and 5.....	19.2	Less than 0.01.
2 and 6.....	7.2	Less than 0.10.
5 and 9.....	11.1	Do.
6 and 10.....	5.8	0.10.

¹ As an aid in evaluating the data, the significance of certain critical differences has been tested by means of the statistical treatment outlined by Fisher (3, *sec. 24.1*) for determining the degree of significance to be assigned to differences between means. (Many of the differences in this paper are so great as to be obviously significant, while others are so slight as to be evidently without meaning; the statistical tests have been used chiefly with border-line cases, and have been worked out from the original counts of the random samples of which the figures given in the fourth column are the averages.) The results of these statistical tests are given in connection with Tables 2, 3, and 4. "P." indicates the probability that the difference in question could have occurred in the course of random sampling of the same lot of material. P. values greater than 0.05 ordinarily indicate that the difference in question can not be considered significant. As the value of P. drops to 0.02 or 0.01, an increasing degree of confidence may be placed in the results.

By the winter of 1926-27 a few growers were doing away with the use of soap and were emulsifying the raw oil in the spray tank with freshly made Bordeaux mixture. A few were also using the soap-oil emulsion with copper sulphate solution instead of with the usual Bordeaux mixture of copper sulphate and lime. These combinations were included in the tests of that winter, which were made on peach, the materials being applied with the hand outfit. Some of the oil concentrations were made as low as 0.6 per cent, and for the first time the proportion of oil present in the spray mixture was checked by the modified Babcock test. The data from this series of tests are given in Table 3, and have also been used in the preparation of the curves in Figure 4.

TABLE 3.—*Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., winter of 1926-27*

(Oil No. 1 used throughout)

Plot No.	Method of emulsification	Strength of Bordeaux mixture	Strength of oil	Scales counted	Scales dead	Efficiency of spray
			<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>	<i>Per cent</i>
1.....	Soap, heat.....	None.	0.6	1,021	98.8	96.1
2.....			1.2	1,001	99.2	97.4
3.....			1.8	1,000	100.0	100.0
4.....			.6	1,000	98.2	94.2
5.....	do.....	1-0-50	1.2	1,000	98.5	95.1
6.....			1.9	1,000	100.0	100.0
7.....			.7	1,068	98.8	96.1
8.....			1.0	1,011	99.5	98.4
9.....	do.....	½-½-50	1.7	1,000	99.9	99.7
10.....			.8	1,000	91.4	72.2
11.....			1.2	1,000	95.5	85.4
12.....			1.8	1,000	99.1	97.1
13.....	Tank mix.....	4-6-50	.7	1,000	84.6	50.2
14.....			1.1	950	84.4	49.5
15.....			1.6	1,026	96.8	89.6
16.....	Check, no treatment.....			4,739	69.1	-----

TABLE 3.—Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., winter of 1926-27—Continued

SIGNIFICANCE OF DOUBTFUL DIFFERENCES¹

Plots compared	Arithmetic difference	P. (probability that the difference was accidental)
2 and 11.....	12.0	Less than 0.01.
2 and 15.....	7.8	Do.
3 and 12.....	2.9	Approximately 0.05.

¹ See footnote to Table 2.

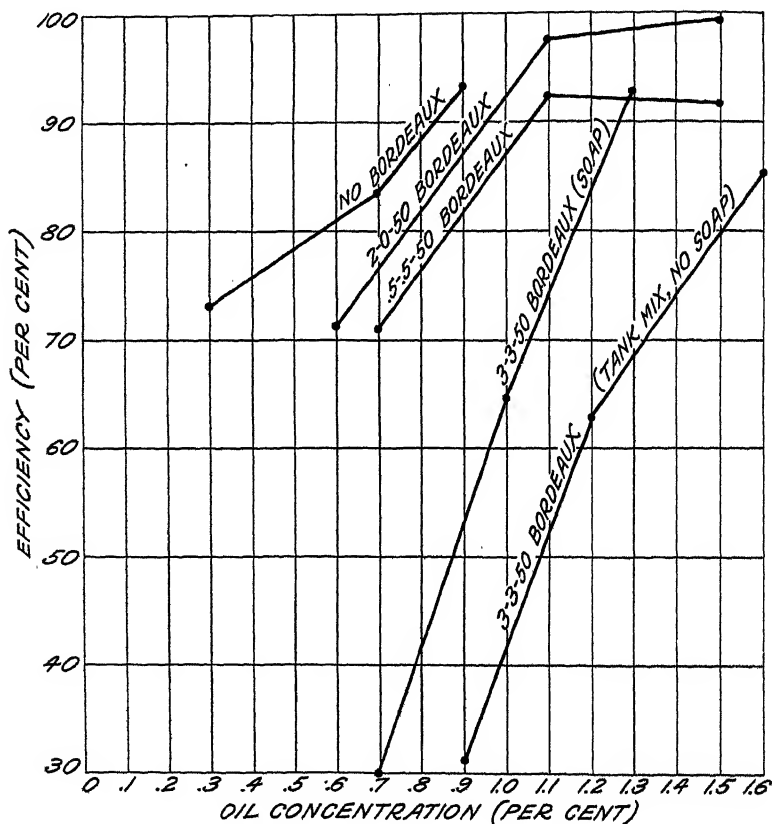


FIGURE 3.—Influence of Bordeaux mixture on the effectiveness of oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., winter of 1927-28. (See Table 4, oil No. 2)

One more repetition of this series of tests was made during the winter of 1927-28, with some concentrations of oil a little lower than those used the previous season. Applications were made to peach with a power outfit. All but one of the tests were in duplicate with the exception that a different oil was used in the second case. The data from this series are presented in tabular form in Table 4. The results of the tests in which oil No. 2 was used are plotted in Figure 3.

TABLE 4.—*Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., winter of 1927-28*

Plot No.	Oil used	Method of emulsification	Strength of Bordeaux mixture	Strength of oil	Scales counted	Scales dead	Efficiency of spray
				<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>	<i>Per cent</i>
1.....	Oil No. 1.....	Soap, heat.....	None.	0.4	1,000	94.6	87.3
2.....				.9	1,500	97.7	94.6
3.....				1.5	1,350	96.4	91.5
4.....				.6	1,000	86.3	67.6
5.....				1.0	1,000	97.2	93.4
6.....	do.....	do.....	½-½-50	1.2	1,000	97.8	94.8
7.....	do.....	do.....	3-3-50	.5	1,000	86.4	67.9
8.....				.8	1,000	89.0	74.1
9.....				1.2	1,000	97.3	93.6
10.....				.8	1,000	89.6	75.5
11.....				1.0	1,000	92.9	83.3
12.....	do.....	Tank mix.....	3-3-50	1.5	1,000	96.1	90.8
13.....				.3	1,000	88.6	73.1
14.....				.7	1,000	93.0	83.5
15.....				.9	1,000	97.1	93.2
16.....				.6	1,000	87.8	71.2
17.....	do.....	do.....	2-0-50	1.1	1,000	98.9	97.4
18.....	do.....	do.....	½-½-50	1.5	1,000	99.6	99.1
19.....				.7	1,000	87.7	71.0
20.....				1.1	1,000	96.7	92.2
21.....				1.5	1,450	96.4	91.5
22.....				.7	1,000	70.3	30.0
23.....	do.....	do.....	3-3-50	1.0	1,000	85.0	64.6
24.....				1.3	1,000	96.9	92.7
25.....				.9	1,000	70.8	31.1
26.....				1.2	1,650	84.2	62.7
27.....				1.6	1,000	93.7	85.1
28.....	Check.....		2,000	57.6

SIGNIFICANCE OF DOUBTFUL DIFFERENCES¹

Plots compared	Arithmetic difference	P. (probability that the difference was accidental)
1 and 7.....	19.4	Less than 0.01.
2 and 11.....	11.3	Do.
14 and 19.....	12.5	Less than 0.02.
21 and 27.....	6.4	Approximately 0.20.

¹ See footnote to Table 2.

The date for all of the tests in which oil No. 1 was used, without Bordeaux mixture and with full-strength Bordeaux mixture (Tables 2, 3, and 4), have been combined in plotting the curves shown in Figure 4. Since in all cases the oil reached practically its full toxicity at a concentration of 2 per cent, the graph shows the results only as far as this dilution. The usual method of least squares has been used in plotting the curves.

Preliminary experiments to determine the influence of Bordeaux mixture on one of the well-known miscible oils seemed to show a trend similar to that obtained with lubricating-oil emulsion, but these tests were not sufficiently extensive or conclusive to warrant presenting them in detail.

DISCUSSION OF RESULTS

It is evident from the data here presented that the effectiveness of lubricating oil-soap emulsion at low concentrations is reduced by the addition of ordinary strengths of Bordeaux mixture. In all

dormant tests in which the oil concentration was 1.3 per cent or less, a significant difference has appeared. The curves plotted in Figure 4 indicate that this effect may not entirely disappear until the oil concentration reaches 1.7 or 1.8 per cent, but the data available are not sufficient to establish the exact point where the differences lose their significance.

When emulsified in the tank by Bordeaux mixture, instead of by the usual soap process, the oil appeared in two series of dormant tests to suffer a further reduction in efficiency, whereas in a third series its efficiency appeared to be unaffected. The evidence therefore suggests that this process of emulsification renders the oil less

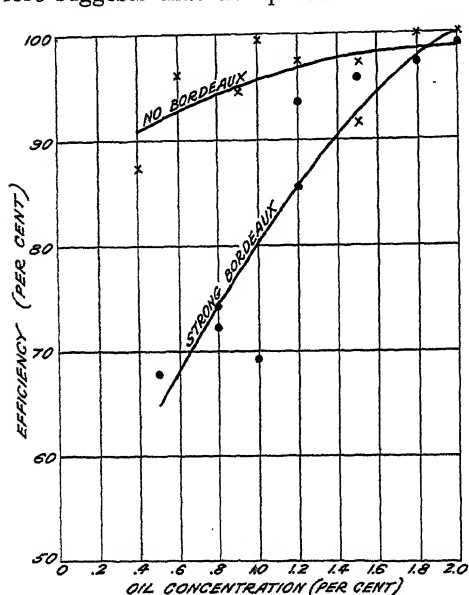


FIGURE 4.—Influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., 1925-1928. The curves were made from data brought together from all dormant spray tests in which oil No. 1 was used. (See Tables 2, 3, and 4)

sprays, and the differences were evident with concentrations of oil up to 2 per cent. The consistent replication of results through seven repetitions of substantially the same tests during two seasons gives the data undoubted significance.

POSSIBLE CAUSE OF REDUCTION IN EFFECTIVENESS

Although the writers have not determined experimentally in just what way Bordeaux mixture acts on the oil in the diluted spray, the following quotations from DeOng, Knight, and Chamberlin (6, p. 362-363) offer a plausible explanation of the loss of efficiency:

Some emulsifiers, such as lime or kaolin and other earths, are capable of absorbing considerable amounts of oil, as well as emulsifying them. This is particularly pronounced and important when the emulsifier or spreader is used in large quantities. All such absorbed oil is unavailable for liberation as a free

effective, although the extent of this reduction can be determined only by further tests.

When a weak Bordeaux mixture was used with the oil spray, as for the purpose of maintaining a stable emulsion with hard waters, slightly significant reductions in effectiveness occurred in some tests but failed to appear in others. The addition of copper sulphate solution appears to have a similar slightly detrimental influence, although more extensive tests are needed before definite conclusions can be drawn.

In the tests conducted during the summer the influence of Bordeaux mixture on the effectiveness of the oil was more marked than in the dormant

liquid and constitutes, therefore, a permanent loss—assuming, of course, that free oil is the effective agent. * * *

Lime, by its absorptive capacity, tends to prevent the oil from coming into direct contact with the object sprayed, and hence serves as an inhibiting factor.

The writers believe that the Bordeaux mixture acts in a mechanical way, absorbing a certain volume of oil, thus preventing its penetration into the insect.

PRACTICAL APPLICATION

In actual practice oil is ordinarily used at a higher concentration than is necessary for perfect control with heavy and thorough application. The excess oil offsets in some measure the lightness of many of the commercial applications, although it can not make up for the missing of entire portions of trees. If the oil is used at a concentration not sufficiently above the exact point of full efficiency it is obvious that the addition of Bordeaux mixture may reduce its effectiveness to a point where incomplete control will result. In terms of orchard practice, the addition of full-strength Bordeaux mixture would warrant an increase in the oil content of the spray by 0.5 to 1 per cent of the total volume of diluted material. For example, if in dormant spraying without Bordeaux mixture, or with a very dilute Bordeaux mixture, 2 per cent of actual oil has been found to be barely enough to control the scale, the addition of a heavy Bordeaux mixture should be accompanied by an increase in the oil content to 2.5 or 3 per cent.

With trees in foliage, the oil content of the spray mixture can not always be increased with safety. In case an emergency necessitates spraying with oil for scale control in the summer, the oil should be applied separately rather than in combination with one of the regular applications of Bordeaux mixture.

SUMMARY

As oil sprays for the control of insects are sometimes combined with fungicides, an investigation has been made of the comparative efficiency of the oil emulsion in such combinations.

Lubricating-oil emulsion was found to be reduced in efficiency in the control of the San Jose scale by the addition of Bordeaux mixture at usual strengths (2–3–50 to 4–6–50). This reduction appears in dormant spraying at low oil concentrations, and practically disappears, under the conditions of these experiments, when the oil content reaches 1.5 per cent. In summer spraying, this reduction is very marked with oil concentrations up to 2 per cent.

In practical dormant spraying this renders desirable a moderate increase in the oil content of lubricating-oil emulsion whenever full-strength Bordeaux mixture is used with it. Oil applications in the summer for emergency control should be made separately rather than in combination with one of the regular Bordeaux sprays.

LITERATURE CITED

- (1) ABBOTT, W. S.
1925. A METHOD OF COMPUTING THE EFFECTIVENESS OF AN INSECTICIDE. *Jour. Econ. Ent.* 18: 265–267.
- (2) ACKERMAN, A. J.
1923. PRELIMINARY REPORT ON CONTROL OF SAN JOSE SCALE WITH LUBRICATING-OIL EMULSION. U. S. Dept. Agr. Circ. 263, 18 p., illus.

-
- (3) FISHER, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 p. Edinburgh and London.
- (4) GRIFFIN, E. L., and RICHARDSON, C. H.
1926. A FIELD METHOD FOR DETERMINING THE OIL STRENGTH OF SPRAYS. *Jour. Econ. Ent.* 19: 522-525.
- (5) HARMAN, S. W.
1927. THE PEACH COTTONY SCALE. N. Y. State Agr. Expt. Sta. Bul. 542, 19 p., illus.
- (6) DE ONG, E. R., KNIGHT, H., and CHAMBERLIN, J. C.
1927. A PRELIMINARY STUDY OF PETROLEUM OIL AS AN INSECTICIDE FOR CITRUS TREES. *Hilgardia* 2: [351]-384, illus.
- (7) PICKERING, S. U.
1907. EMULSIONS. *Jour. Chem. Soc. [London]* 91: 2001-2021, illus.
- (8) YOTHERS, W. W.
1918. SPRAYING FOR THE CONTROL OF INSECTS AND MITES ATTACKING CITRUS TREES IN FLORIDA. U. S. Dept. Agr. Farmers' Bul. 933, 38 p., illus. (Revised 1922, 44 p., illus.)

STUDIES OF VITAMIN C IN FRESH AND CANNED TOMATOES¹

By BERTHA CLOW and ABBY L. MARLATT, *Department of Home Economics, University of Wisconsin*²

INTRODUCTION

The value of vitamin C as an antiscorbutic has been so well established that it has become almost axiomatic to recommend raw fruit juice high in this vitamin for feeding infants and young children. Orange juice is still looked upon as the standard, but tomato juice is now being widely substituted. For this reason it becomes important to know to what extent, if at all, the vitamin-C content of canned tomatoes is affected by the different methods used in canning.

The experiments reported in this paper were carried out to answer the following questions: Are canned tomatoes as reliable as fresh tomatoes as a source of vitamin C? How long after canning are home-canned tomatoes a satisfactory source of this vitamin? If tomatoes are picked green and allowed to ripen at room temperature, will they still be rich in vitamin C? Is the vitamin-C content of greenhouse-ripened tomatoes as high as that of field-ripened tomatoes? Is the commercial method of coloring tomatoes in an atmosphere of ethylene destructive to vitamin C? Does the usual household method of making green-tomato pickles destroy vitamin C? The answers to these questions are found in this report of experimental studies carried on in the home economics research laboratory of the University of Wisconsin from 1925 to 1928.

EXPERIMENTAL PROCEDURE

The recovery method of study was used throughout the investigation. This method was preferred to the protection method, which is most frequently reported for vitamin-C work, because tomatoes of all the types studied were not available for a full 60-day feeding period. The recovery method used was as follows: Guinea pigs were fed a scurvy-producing ration plus cabbage ad libitum until they reached approximately 250 grams in weight. Then the cabbage was taken away and the scurvy ration alone was fed until positive scurvy symptoms were noted; that is, either a "jerky run" or swollen wrists. The tomato juice was then fed, and the feeding was continued for 16 days. On the seventeenth day the guinea pig was killed and an autopsy performed.

On autopsy the following conditions were looked for: Hemorrhage and swelling of the wrists or elbows of the forelegs and also below the hip joint of the hind legs, hemorrhage or "beading" at the costochondral juncture of the ribs, loose teeth, and any possible hemorrhages on the abdominal organs or on the lining of the abdominal cavity.

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² The authors wish to express their appreciation to Helen T. Parsons and Ella Woods for their guidance in this problem and to Ina Stevenson for her assistance in the care and feeding of the animals used.

The scurvy-producing ration is a modification of that used by Cohen and Mendel³ and also by Parsons and Reynolds.⁴ The ration consists of the following: Soybean flour, 1,620 gm.; dried yeast, 120 gm.; purified casein, 105 gm.; calcium lactate, 60 gm.; sodium chlorate, 60 gm.; filter paper, 40 gm.; and butterfat, 100 gm. The soybean flour is a commercial product, the preparation of which involves heating. The yeast also is a commercial product and is a reliable source of the vitamin-B complex. The casein was purified by soaking for a week in water slightly acidified with glacial acetic acid (about 5 c. c. per 6 quarts of tap water). This water was changed every day. The filter paper was cut into small pieces and beaten in distilled water until a fine pulp was obtained. This was poured upon the mixed dry ingredients and the mass rubbed until evenly mixed. When dry it was ground and thoroughly mixed with the melted butterfat.

The tomatoes used in these studies were obtained for the most part from the university greenhouse and gardens, though some were received from a local grocery and some from a local commercial greenhouse. They were all either Bonnie Best or a similar variety.

Two methods of canning were employed, (1) the cold-pack and (2) the open-kettle method. By the first method the tomatoes are blanched in boiling water for 3 minutes, the skins removed, and the fruits packed in sterile $\frac{1}{2}$ -pint jars. The jars are then put in a boiling-water bath for 20 minutes and the seal completed. By the second method the tomatoes are blanched, the skins removed, and the fruit cooked in an open kettle for 20 minutes and then put into $\frac{1}{2}$ -pint sterile jars and sealed.

The green tomato pickles were prepared according to a common household method as reported in popular cookbooks, except that spices and additional flavors such as onion and pepper were omitted. Sliced tomatoes (8,000 grams) and salt (400 grams) were placed in alternate layers in crockery bowls and allowed to stand 24 hours. At the end of that time the salt liquid was drained off (2,445 grams). To the tomatoes, vinegar and sugar were added and the mixture cooked in the open kettle for a total period of 55 minutes and then packed in $\frac{1}{2}$ -pint sterile jars and sealed.

The ethylene-colored tomatoes were grown in the greenhouse and picked when they were green but full grown. They were colored in a small air-tight chamber (an ice box) to which the proportion of ethylene to cubic-foot volume of the chamber was 1 part to 1,000. From four to eight days were required to color the tomatoes. This is perhaps a little longer than is necessary commercially, but the tomatoes showed no sign of coloring when they were put into the chamber, and they were left long enough to develop a very good red color.

EXPLANATION OF TEXT FIGURES

The initial growth period between the time that cabbage was removed from the diet and scurvy symptoms were evident is not included in the curves. The short crossline indicates the point at which a definite case of scurvy was diagnosed and the 16-day tomato feeding

³ COHEN, B., and MENDEL, L. B. EXPERIMENTAL SCURVY OF THE GUINEA PIG IN RELATION TO THE DIET. *Jour. Biol. Chem.* 35: 425-453. 1918.

⁴ PARSONS, H. T., and REYNOLDS, M. S. THE DEPLETION OF VITAMIN C IN THE LIVER OF THE GUINEA PIG ON A SCORBUTIC RATION. *Jour. Biol. Chem.* 59: 731-736, illus. 1924.

period begun. The following system of letters indicates the autopsy findings at the end of the recovery period:

- A, scurvy cured.
- A-, scurvy practically cured.
- B+, scurvy considerably cured.
- B, scurvy moderately cured.
- B-, scurvy very slightly cured.
- C, scurvy symptoms very marked.

It should also be added that the weight curves of all animals on the same dosage of tomato although available are not included in cases where there are several animals. It was chosen rather to represent what would be considered average or typical results. The number of guinea pigs on each dosage of each kind of tomato is included in each graph. This number totals 107 exclusive of the controls. With practically each kind of tomato, however, both higher and lower dosages than those presented were actually fed. It seemed necessary to include data only on the "borderline" doses, i. e., the minimum dosage to bring about approximately complete recovery in 16 days.

DISCUSSION OF RESULTS

The question of the relative importance of the character of the weight curve and the degree of recovery as shown by the autopsy must be considered. As a general rule resumption of growth accompanies recovery from the scorbutic condition. However, when one is determining the borderline dosage on which recovery

is obtained there is very often resumption of growth but only partial recovery shown when autopsy is performed at the end of 16 days. These are doubtless cases where a feeding period of more than 16 days would have brought about complete recovery, but the 16-day recovery period being taken as a standard for comparison, the authors believe that the degree of recovery as shown by the autopsy is a more important criterion than the character of the weight curve.

FIELD-RIPENED RAW TOMATOES

The curves and degree of recovery as shown in Figure 1 indicate that a 2-gram daily dosage of raw tomatoes ripened in the field does not bring about recovery, but that a 3-gram dosage does bring about practically complete recovery. Six animals were fed the former and eight the latter dosage. Throughout these studies the field-ripened raw tomatoes are used as the standard of comparison, and 3 grams daily is considered the minimum recovery dosage. All comparisons are made on this basis.

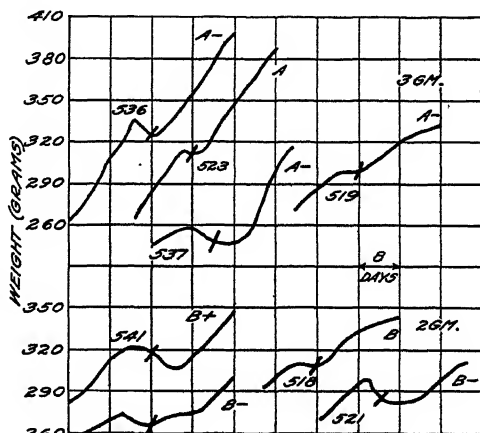


FIGURE 1.—Growth and recovery of guinea pigs on 2 and 3 gram daily doses of field-ripened raw tomatoes fed as an antiscorbutic. The system of lettering on this and the other figures is explained in the text

FIELD-RIPENED CANNED TOMATOES FED WITHIN NINE MONTHS AFTER CANNING

The results shown in Figure 2 for 4 of the 10 guinea pigs used very clearly indicate that a 3-gram dosage of cold-pack and water-bath processed tomatoes fed within nine months after canning brings about practically complete recovery. Thus it seems that a storage period of nine months does not lessen the vitamin-C content of tomatoes canned by the cold-pack method.

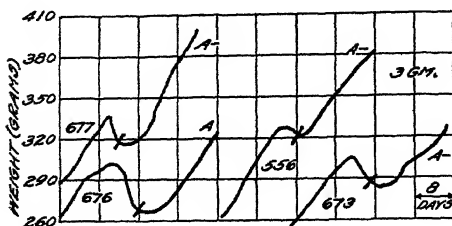


FIGURE 2.—Growth and recovery of guinea pigs on a 3-gram daily dosage of field-ripened tomatoes canned by the cold-pack, water-bath process and fed within nine months as an antiscorbutic. The tomatoes were from the same lot as those mentioned in Figure 1

by the results in Figure 3, which indicate that a 3-gram dosage of tomatoes canned by this method can not compare in vitamin-C content with a like dosage of tomatoes canned by the cold-pack method. In fact, even 4 grams did not always give complete recovery, although this dosage can probably be considered the minimum recovery dosage when this method is used. This loss of vitamin C is quite understandable in view of the well-known instability of vitamin C to oxidation.

FIELD-RIPENED CANNED TOMATOES FED 15 TO 20 MONTHS AFTER CANNING

Field-ripened tomatoes were canned by the cold-pack method in September, 1926. Later that fall and also in the spring of 1927 tomatoes from this lot were fed, and 3 grams was found to be the recovery dosage. (Fig. 2.) When tomatoes from the same lot were fed in the late fall of 1927 and

in the spring of 1928, that is 15 to 20 months after canning, 3 grams would not bring about recovery but a 4-gram daily dosage was necessary. (Fig. 4.) Nine animals were fed the former and six the latter dosage. Why this deterioration in storage did not appear in 8 or 9 months but was evident in 15 months can not be explained, except possibly by the fact that the summer months intervened. The canned tomatoes were not kept in a cool room but in the laboratory, where the summer temperature was often higher than 70° F. There were no signs of spoilage, however, when the jars were opened.

This loss of vitamin C during long storage of the canned product is further shown by the field-matured but green canned (cold-pack)

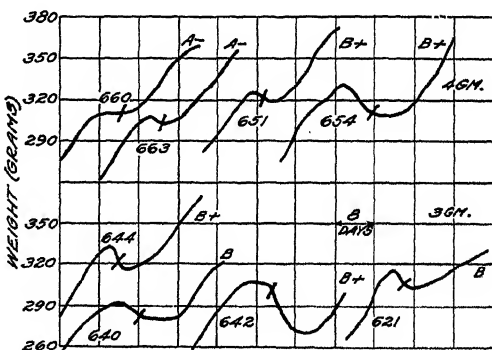


FIGURE 3.—Growth and recovery of guinea pigs on 3 and 4 gram doses of daily field-ripened tomatoes canned by the open-kettle process and fed within nine months as an antiscorbutic. The tomatoes were from the same lot as those mentioned in Figure 1

tomatoes. Within the first nine months after canning 5 grams was the recovery dosage. (Fig. 5, B.) Two animals were fed raw, green, field-matured tomatoes, and six raw greenhouse (full-grown) tomatoes with the same result; five were fed the full-grown green canned tomatoes. But 15 to 20 months later (the summer months having intervened) the results in Figure 6 indicate that 5 grams would not bring about the same degree of recovery.

FIELD VERSUS GREENHOUSE TOMATOES

Greenhouse tomatoes left on the vines until thoroughly ripe were picked in one lot and kept in an electric refrigerator during the feeding period from July 13 to August 2, 1928. The records in Figure 7 show that a 3-gram dosage gave moderate recovery from scurvy and a 4-gram dosage gave approximately complete recovery. Nine animals were fed the former and four the latter dosage. By comparing these results with the standard of 3 grams for field-ripened tomatoes, it appears that greenhouse-ripened tomatoes are not quite so potent a source of

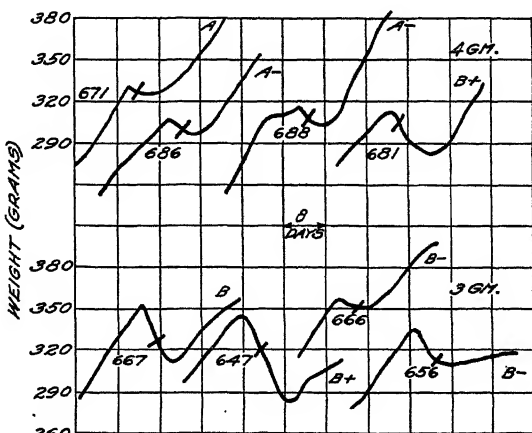


FIGURE 4.—Growth and recovery of guinea pigs on 3 and 4 gram daily doses of field-ripened tomatoes canned by the cold-pack, water-bath process, and fed 15 to 20 months later as an antiscorbutic

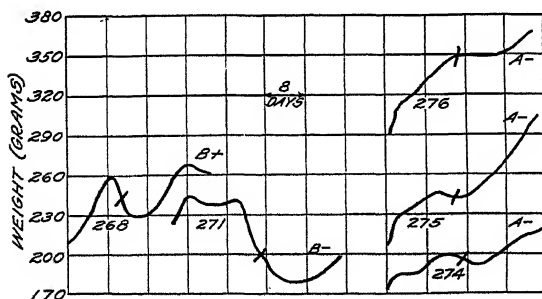


FIGURE 5.—Growth and recovery of guinea pigs on a 5-gram daily dosage of field-matured green tomatoes raw (left) and also canned by the cold-pack, water-bath process, and fed within nine months as an antiscorbutic (right)

vitamin C as field-ripened tomatoes. The field tomatoes were also picked in one lot and kept in the electric refrigerator during the feeding period (August 29 to September 23, 1927), so that the question of different handling of the tomatoes does not enter in as an explanatory factor.

EFFECT OF ETHYLENE COLORING⁵

A recent development in the commercial world is the use of ethylene for ripening or coloring certain fruits and vegetables. Work done at

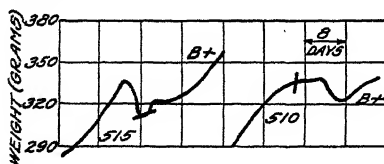


FIGURE 6.—Growth and recovery of two guinea pigs on a 5-gram daily dosage of field-matured green tomatoes canned by the cold-pack, water-bath process, and fed 15 to 20 months later as an antiscorbutic. The tomatoes were from the same lot as those mentioned in Figure 5

the laboratory as described previously. After they were colored they were placed in the electric refrigerator at about 42° F. The results as shown in Figure 8 indicate that 4 grams is the recovery dosage, seven animals being fed the 3 grams and four the 4 grams dosage. Thus coloring in an atmosphere of ethylene does not entirely prevent the development of vitamin C, and moreover the vitamin seems to develop to the same extent as though the tomatoes were allowed to ripen on the vine in the greenhouse.

GREENHOUSE TOMATOES ALLOWED TO RIPEN IN A DARK ROOM

Full-grown but green tomatoes grown in the greenhouse were picked on April 17, 1927, and put into a dark closet at room temperature. On May 18, 1927, the tomato-feeding period was started, but it was June 9 before all the guinea pigs had received the last dose. In the meantime the tomatoes had become very soft, too soft in fact for table use. It was found in an

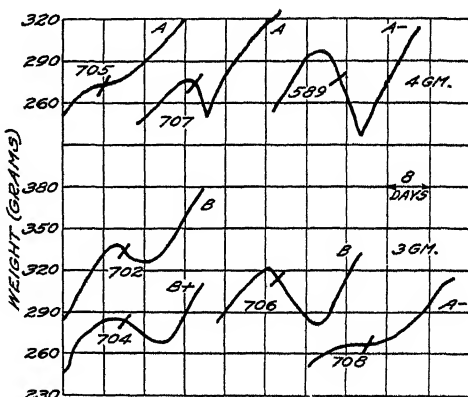


FIGURE 7.—Growth and recovery of guinea pigs on the 3 and 4 gram daily doses of greenhouse tomatoes which had been allowed to become thoroughly ripe on the vine and were then fed as an antiscorbutic

⁵ Since this paper was put into final form for publication, the following article regarding the effect of ethylene ripening of tomatoes on the vitamin A, B, and C content has appeared: HOUSE, M. C., NELSON, P. M., and HABER E. S. THE VITAMIN A, B, AND C CONTENT OF ARTIFICIALLY VERSUS NATURALLY RIPENED TOMATOES. Jour. Biol. Chem. 89: 495-504, illus. 1929. House, Nelson, and Haber showed that tomatoes which had been picked when green and then air-ripened had the same vitamin-C content as similar tomatoes ethylene-ripened, but that both the air-ripened and ethylene-ripened tomatoes were less potent in vitamin C than the vine-ripened (greenhouse) tomatoes. Thus, they show that vitamin C does not develop in the tomato to the same extent after it is picked from the vine that it does when it is allowed to ripen on the vine. House, Nelson, and Haber used only the 4-gram level in feeding, which is high, as shown in this paper, where the minimum recovery dosage is reported in each case. By the latter method the results seem to show that vitamin C develops practically to the same extent whether the ripening takes place on the vine, in a dark room, in a light room, or in an atmosphere of ethylene. The present studies confirm those of House, Nelson, and Haber in showing that there is an increase of vitamin C in the ripening of tomatoes and also that ethylene ripening may not be destructive to vitamin C in tomatoes, though it may to a slight extent inhibit its development.

experiment in which five animals were used that 3 grams of these tomatoes gave approximately complete recovery (fig. 9), thus showing that vitamin C develops after the tomato has been severed from the vine and allowed to ripen in a dark room.

VITAMIN-C CONTENT OF TOMATOES AT DIFFERENT STAGES OF MATURITY

As a corollary to the other studies, data were obtained on the vitamin-C content of tomatoes at different stages of maturity.

Although weight curves are not included, an attempt was made to determine a recovery dosage when small, immature, green tomatoes about 1 inch in diameter from both field and greenhouse were used.

In preparing these tomatoes for feeding it was necessary first to rub them over a grater or put them through a grinder and then obtain the juice by putting the pulpy material into a cheesecloth bag and squeezing it. Because of the pungent taste, it was very difficult to get the animals to take this juice.

Five and seven gram doses failed to bring about recovery, and even the animal on the 10-gram dosage died after 9 days' feeding of raw, green, immature tomato. However, when immature field tomatoes were canned by the usual cold-pack method and fed to three guinea pigs in 10-gram doses all recovered in the 16-day period.

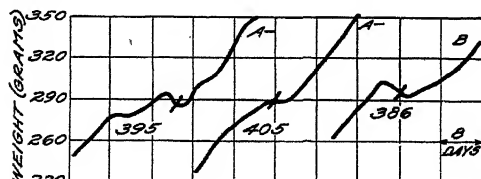


FIGURE 9.—Growth and recovery of guinea pigs on a 3-gram daily dosage of greenhouse tomatoes, picked when mature but green, and allowed to ripen in a dark closet at 70° F. before being fed as an antiscorbutic

tomato is the recovery dosage this seems to show that vitamin C in tomatoes develops with the maturing process.

EFFECT OF SOFTENING ON THE VITAMIN C CONTENT OF TOMATOES

In a preliminary study of the effect of softening on the vitamin-C content of tomatoes, both fresh and canned green fruits were fed. It

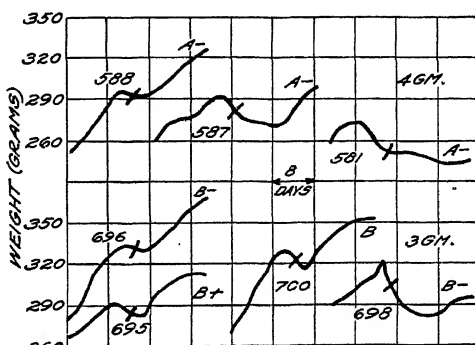


FIGURE 8.—Growth and recovery of guinea pigs on 3 and 4 grams daily doses of greenhouse tomatoes picked when mature, but green, and then allowed to develop a good, red color in an atmosphere of ethylene gas before being fed as an antiscorbutic. The tomatoes were the same lot as those mentioned in Figure 7

In feeding the mature but green canned tomatoes the minimum recovery dosage is 5 grams. (Fig. 5, right.) As data have been presented showing that 3 grams of the mature ripe raw or cold-pack canned (field) to-

* The writers show that canned green tomato is more effective in feeding (for vitamin C) than the raw, and they wish to call attention to the following publication on the vitamin-A content of asparagus: CRIST, J. W., and DYE, M. THE ASSOCIATION OF VITAMIN A WITH GREENNESS IN PLANT TISSUE. II. THE VITAMIN A CONTENT OF ASPARAGUS. Jour. Biol. Chem. 81: 525-532, illus. 1929. In the words of Crist and Dye. " * * * it is relevant to infer that the poor quality of bleached asparagus as food for the animal may not be due alone to vitamin A deficiency but also to an over-abundance of deleterious chemical compounds. This conclusion has some additional support in the fact that cooking the white asparagus improved its nutritive value to some extent; a result which could have been due to the effect of the cooking process on the chemical constituents of the tissue" (p. 531).

was found that 5 grams of canned field-matured green tomato was the recovery dosage, but that 5 grams of the raw field-matured green tomatoes would not bring about the same degree of recovery. (Compare left and right fig. 5.) Thus the canned green tomatoes seem better than the raw.

To determine the effect of softening on the vitamin-C content, raw field-matured green tomatoes were wrapped in paper and stored

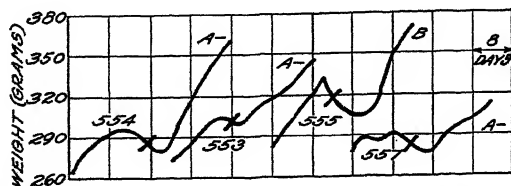


FIGURE 10.—Growth and recovery of guinea pigs fed a 5-gram daily dosage of field-matured green tomatoes, stored for three weeks at 42° F., and then fed as an antiscorbutic

in a large refrigerator in one of the university buildings and allowed to remain there for three weeks. At the end of that time it was found that they had softened, and when fed to six guinea pigs at a 5-gram level they gave approximately complete recovery. (Fig. 10.) As mentioned above, 5 grams of raw field-matured green tomatoes fresh from the vine would not bring about recovery, and 5 grams of this same kind of green tomato canned by the cold-pack method did bring about recovery. As these green, raw, but stored tomatoes gave the same recovery as green canned tomatoes the difference in vitamin-C value after softening but not coloring is of interest.

Some of these stored tomatoes which had begun to turn pink were allowed to color further at room temperature (70° F.). Although they never developed a red color like that of the field-ripened tomatoes or the tomatoes allowed to ripen at room temperature without previous storage, it required only slightly more than a 3-gram dosage to bring about recovery. Four animals were fed the 3 and three the 4 gram dose. (Fig. 11.)

As previously stated, when greenhouse tomatoes were picked green and allowed to ripen in a dark room only a 3-gram dosage was required to bring about recovery, whereas when greenhouse tomatoes were picked from the vines when ripe a 4-gram dosage was required. The tomatoes which had been allowed to ripen after being picked were very soft when fed. Possibly in the case of the stored tomatoes the factor of loss of moisture with greater concentration of vitamin C per unit of weight should not be overlooked. However, the fact that canned green tomatoes are more effective than raw green tomatoes can hardly be explained on the basis of loss of moisture.

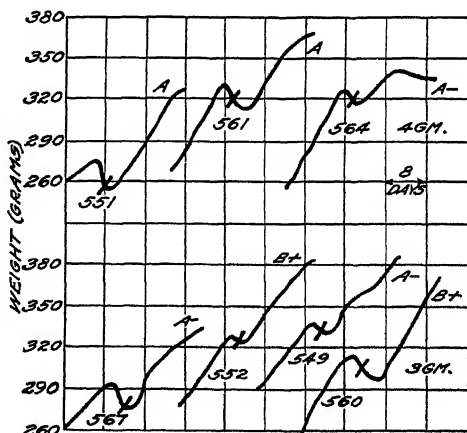


FIGURE 11.—Growth and recovery of guinea pigs on 3 and 4 gram daily doses of field-matured green tomatoes, stored for three weeks at 42° F. and then allowed to turn pink at 70° F. before being fed as an antiscorbutic. The tomatoes were the same lot as those mentioned in Figure 10

EFFECT OF PICKLING ON VITAMIN C IN GREEN TOMATOES

Field-matured green tomatoes were made into pickles as described previously. Some tomatoes from the same lot were canned by the usual cold-pack method and used as vitamin C controls in this study on the effect of the pickling process. Figure 5, B, shows that there is a considerable amount of vitamin C in these canned green tomatoes, as 5 grams is the minimum recovery dosage. Cooked and canned green tomato pickles were fed at levels up to and including 10 grams, but, as Figure 12 indicates, at no time was a recovery level reached. It is therefore apparent that the vitamin-C content of green tomato pickles is negligible.

SUMMARY

The recovery type of experiment was used to study the vitamin-C content of tomatoes under varying conditions as listed below. The recovery method consisted in depleting the storage of vitamin C in the guinea pigs so that definite scurvy symptoms would develop and then determining the minimum dosage of tomato that would bring about approximately complete recovery in 16 days.

The recovery dosage of field-ripened raw tomatoes was found to be 3 grams, and this was taken as the standard of comparison.

In these experiments no difference was detected in the potency of field-ripened tomatoes fed raw and those canned by the cold-pack method and fed within nine months; therefore they are believed to be essentially the same in vitamin-C content.

When field-ripened tomatoes canned by the cold-pack method are used 15 to 20 months after canning, there seems to be a slight loss in the original vitamin-C content.

Field-ripened tomatoes canned by the open-kettle method and fed within nine months are not so potent as those canned by the cold-pack method.

Field-matured green tomatoes stored at a temperature of 42° F. for three weeks and then allowed to turn pink at room temperature (70° F.), and also greenhouse-matured green tomatoes allowed to ripen at room temperature without previous storage, seem to be as potent a source of vitamin C as tomatoes ripened on the vines in the field. Both types of tomato were quite soft when fed.

Greenhouse tomatoes allowed to ripen on the vine are not quite so potent a source of vitamin C as field tomatoes ripened on the vine.

Coloring of greenhouse tomatoes in an atmosphere of ethylene does not greatly alter or affect the development of vitamin C.

Vitamin C increases with maturity of the tomato. Ripening or coloring after the tomatoes have been severed from the vine does not seem to alter the development of vitamin C.

Mature green tomatoes canned by the cold-pack method are a more potent source of vitamin C than raw mature green tomatoes.

The vitamin C in green tomato pickles is negligible.

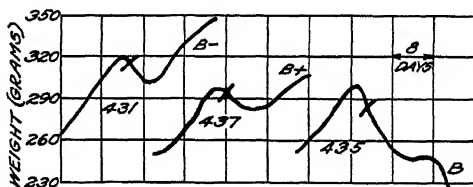


FIGURE 12.—Growth and recovery of three guinea pigs on a 10-gram daily dosage of pickles made from field green tomatoes fed as an antiscorbutic

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PECAN LEAF BLOTCH¹

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INTRODUCTION

Pecan leaf blotch caused by the fungus *Mycosphaerella dendroides* (Cke.) comb. nov. was first collected by the senior writer near Rockport, Ind., in 1923. The following year it was observed at Thomasville, Ga., but at that time it was found only occasionally and was not considered economically important. No extensive survey to determine the exact distribution of the disease has been attempted, but it is now known to occur in many pecan orchards and nurseries in Georgia, Florida, Alabama, Mississippi, and Louisiana. The writers' observations lead them to believe that it is rapidly increasing in importance and is gradually extending its range.

THE DISEASE

Leaf blotch is first found in June on a few of the older leaves of the pecan and may gradually increase until all mature leaves are spotted or killed. On nursery trees it first appears on the lower and older leaves, but as the season advances the others are also attacked. Defoliation progresses upward, till by the 1st of November only a few of the youngest and uppermost leaves remain.

Injuries caused by borers, rosette, general neglect, or any other factor that lowers the vitality of trees predispose them to attack by the leaf-blotch fungus. As is usual with a foliage disease, the damage caused by it can not be estimated accurately. The presence of other leaf-spot fungi, such as *Cercospora caryigenum* (E. and E.) v. Hohnel, *Cercospora fusca* Rand, *Cladosporium effusum* (Wint.) Demaree, and *Phyllosticta caryae* Pk., increases the difficulty of estimating losses. The loss, however, at times is considerable, judged by the extent of the defoliation.

Trees too thickly planted or growing in land that receives no cultivation often lose one-half to three-fourths of their leaves prior to the harvest season on account of leaf blotch. Such heavy defoliation results in nuts of inferior quality and a light crop the year following. Pecan nurseries seem to furnish excellent conditions for infection and for propagation of the fungus. The severity of the attack on nursery-tree foliage may not be due so much to the crowded condition of the trees as to the presence of innumerable infection sources on the ground beneath them. Since cultivation of the nursery ordinarily is

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² The writers wish to express to C. L. Shear, John W. Roberts, and W. W. Diehl, of the Bureau of Plant Industry, their appreciation for helpful suggestions and numerous courtesies extended during the process of the investigation.

not deep enough to cover the previous year's infected leaves, many are left either uncovered or only partially covered, and in these the fungus lives through the winter. Trees growing in good soil, well fertilized and cultivated, are not attacked with the same severity as neglected trees.

The diseased areas show on the upper surface of the leaf first as undefined faint yellow spots which gradually change to dark brown without any clear line of demarcation. The spots on the lower surface are more distinct as they are occupied by a dense growth of erect conidia and conidiophores. (Fig. 1, A.) Later this aerial growth collapses and forms a layer that completely hides the affected host tissues. In mass the color of the aerial fungous structure is at first dark brown, but later, after becoming matted, it appears greenish brown. The spots vary greatly in size, from 1 to 8 mm. in diameter. They may be few in number, but more frequently they are quite numerous, crowded, and coalesce, forming large blotches that may involve a large portion or even the entire lower leaf surface.

During midsummer, black conical pycnidia commonly form on the affected host tissues underneath the mats of conidia and conidiophores. As the season advances the pycnidia increase in number and area and often extend beyond the limits occupied by the aerial hyphae and conidia. Primordia of perithecia develop among the pycnidia during early autumn. The latter structures are not at first easily distinguished from the pycnidia. During the course of the summer the conidia and conidiophores are washed away by rains, thus exposing to view the more conspicuous black pycnidia and immature perithecia. (Fig. 1, B.)

THE CAUSAL FUNGUS

MORPHOLOGY

CONIDIAL STAGE

The conidiophores penetrate the lower epidermis either singly or in small clusters and irrespective of the stomatal openings. They are light to dark brown, erect or decumbent, and may be either simple or branched. (Fig. 2, A and B.) The first-formed conidia are produced near the apical end of conidiophores originating from subepidermal hyphae. Later decumbent hyphae grow over the spots and also bear conidia.

As a rule the conidia are formed singly, but sometimes they are found in short chains of two to three. (Fig. 2, C.) They are subhyaline to light brown, elongate, slightly curved, and 8 to 10 septate; and each segment almost invariably has a large and conspicuous vacuole. The greatest thickness of a conidium is at a point about one-fourth to one-third of its length from the basal end. From the thickest part a conidium tapers toward both ends, but the apical end is drawn out to a more or less attenuated prolongation, giving the conidium a pronounced rostrate appearance. All segments of the conidia, even the hyaline apical ones, may produce germ tubes. (Fig. 2, E.)

PYCNIDIAL STAGE

As stated previously, small black structures are formed during the early development of the spots on the underside of pecan leaflets. These bodies were first considered as spermatogonia, but since there

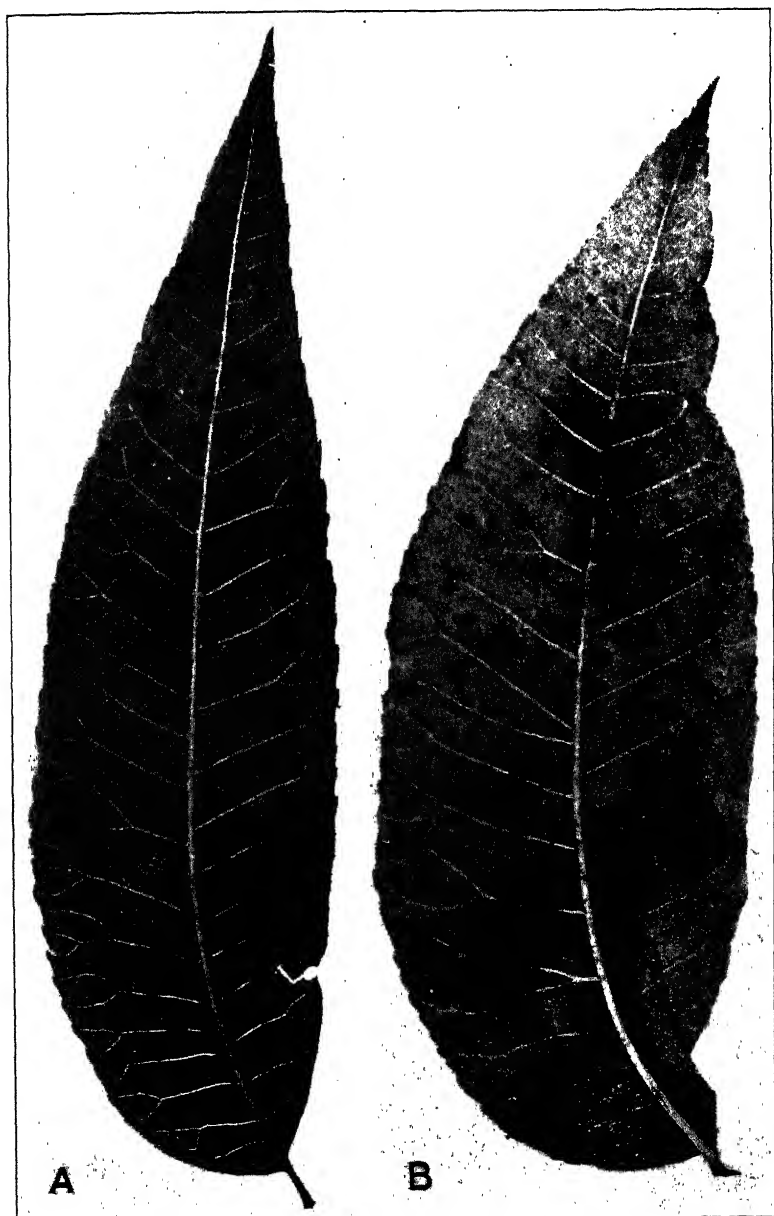


FIGURE 1.—Different stages of pecan leaf blotch on the undersides of leaflets: A, Early stage, showing spots caused by groups of conidia and conidiophores; B, later stage, showing blotched effect caused by groups of pycnidia and immature perithecia

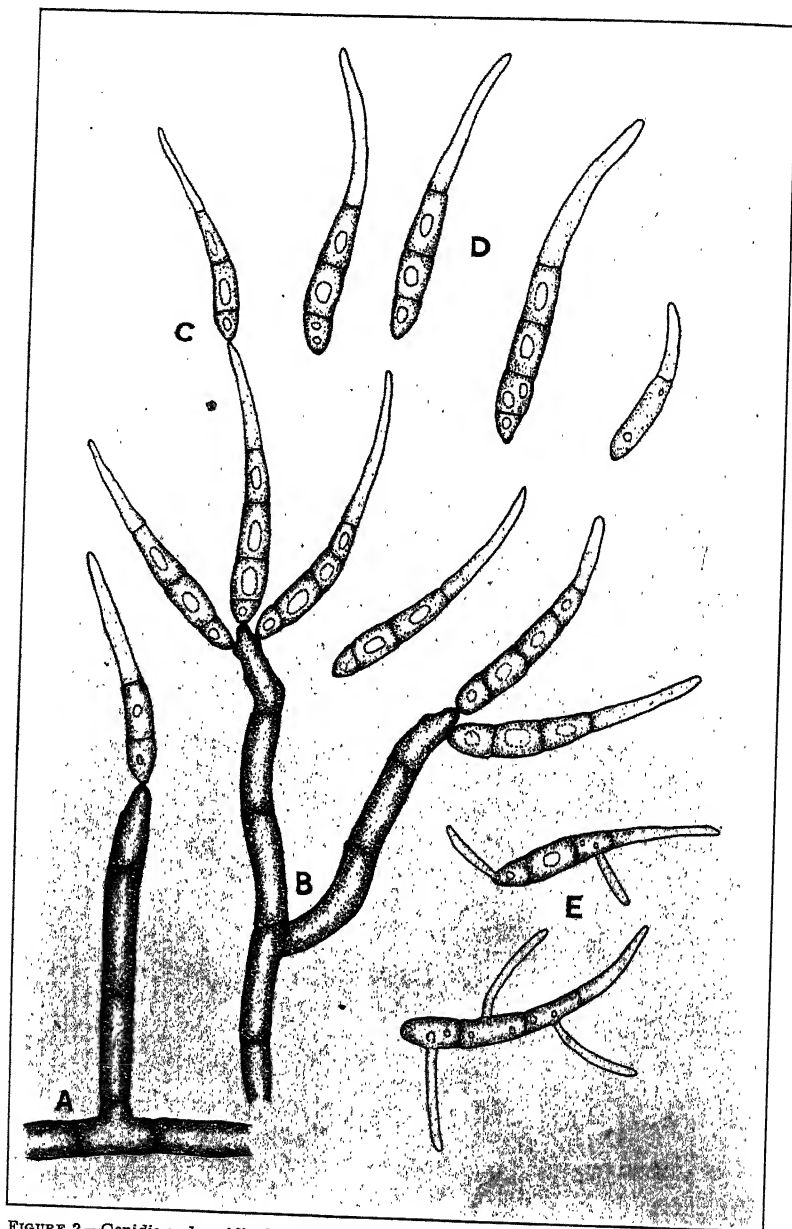


FIGURE 2.—Conidia and conidiophores of the pecan leaf-blotch fungus, *Mycosphaerella dendroides*: A, Conidiophore growing from decumbent hyphae; B, branched conidiophores originating from subepidermal hyphae; C, catenulate conidium; D, representative conidia, showing different shapes and sizes; E, germinating conidia. $\times 825$

is no proof of their functioning as such, it is thought best to refer to them as pycnidia. This spore form may be the same as *Phyllosticta conxerula* described by Bubak (1).³ The spores are of about the same size, and Bubak reported that his *Phyllosticta* was associated with immature perithecia of a *Mycosphaerella*.

The pycnidia, which are at first brown and subepidermal, later break through the epidermis and become black. About one-half to two-thirds of the pycnidium is embedded within the host tissue, out of which it can readily be lifted with a sharp-pointed needle. The pycnidial wall is two to four cells thick. The contents of the pycnidia are composed of many small rod-shaped bodies, 0.5 to 1 μ across and 2 to 4 μ long. (Fig. 3, A.) Repeated attempts to germinate the pycnospores in tap water, distilled water, or in poured plates of various culture media have been uniformly unsuccessful.

The pycnidia mature shortly after they emerge through the epidermis, and irregular cracks or lacerate openings appear in the apical part, thus allowing the contents to emerge as a small waxy mass. Microtome sections of old pycnidia show that many of them are either partially or entirely empty. Conidiophores commonly grow from the exposed parts of the pycnidia, and sections show the basal end of the conidiophores attached to the pycnidial wall.

PERITHECIAL STAGE

The perithecia develop either singly or in groups, principally on the undersides of the green leaves during the latter half of the summer, but they do not mature until the following spring. The groups are circular to irregular in outline and often conform to the shape of the spaces between the leaflet veins. (Fig. 3, B.) A mixture of pycnidia and perithecia often occurs. Almost invariably conidia and conidiophores are found intimately associated with the immature perithecia. In fact, conidiophores often arise from perithecia, and microtome sections show them to be connected with the perithecial body. Young perithecia can not always be distinguished macroscopically from pycnidia, nor always by the aid of a hand lens or a binocular microscope. Ordinarily, however, they are larger, more irregular in form, and frequently have a flat or convex top while immature. Before rupturing the cuticle, and for some time afterwards, they have a shiny appearance. Finally they become dull and rough.

Perithecia at first are embedded within the host tissues, but later they become erumpent. Microtome sections exhibit the very young perithecia as small masses of short thin-walled hyaline cells with no perithecial wall. (Fig. 3, E.) The perithecial wall first begins to form at the apex of the undifferentiated mass which lies next to the lower epidermis of the leaf. (Fig. 3, F.) The cells of the wall form successively toward the base of the young perithecium and finally inclose the mass. The perithecium soon reaches its full size, ruptures the epidermis, and is plainly seen with the naked eye.

A well-developed ostiole is formed, but until the ascospores are mature it is closed by a layer or plug of fungous tissue. (Fig. 4.) As the ascospores approach maturity, the consequent increase in pressure of the asci upon the plug results in the formation of an opening through which the ascospores are ejected, often with force sufficient to

³ Reference is made by number (italic) to "Literature cited," p. 789.

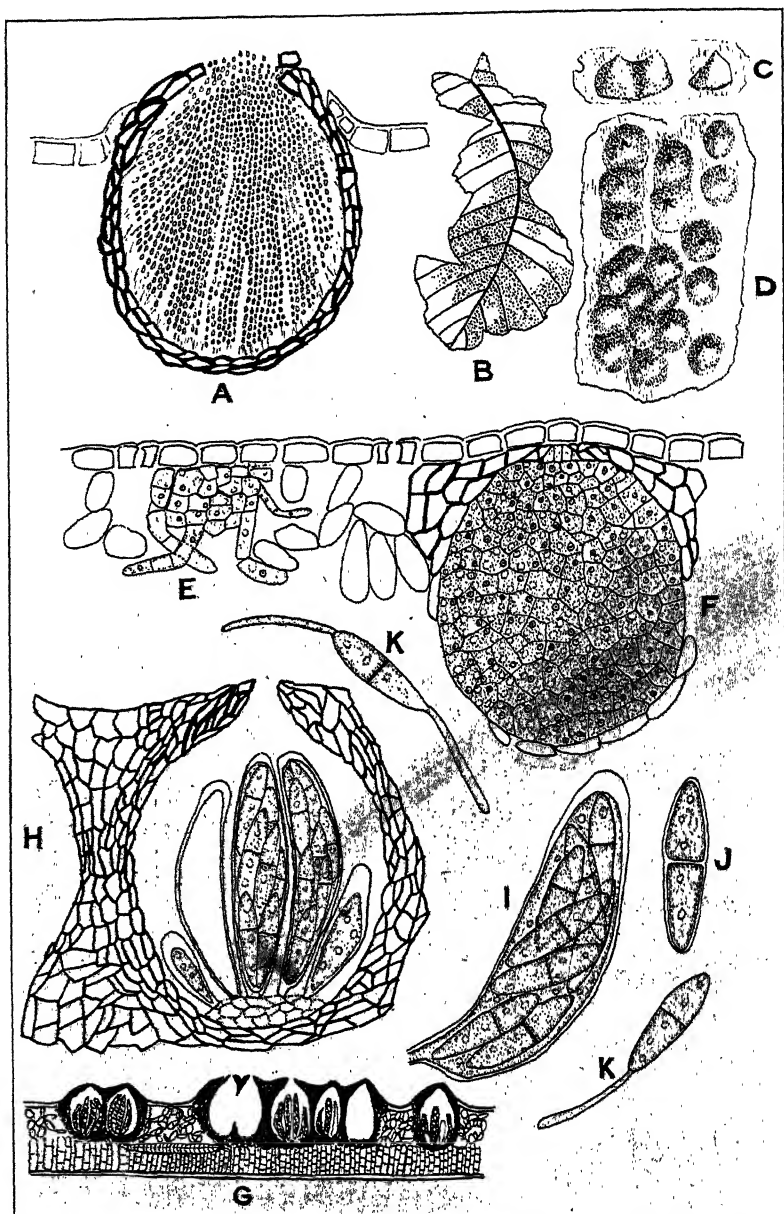


FIGURE 3.—Pycnidial and perfect stages of the pecan leaf-blotch fungus, *Mycosphaerella dendroides*: A, Pycnidium containing spores. $\times 430$. B, Old pecan leaflet with mature perithecia. $\times \frac{1}{2}$. C, D, Enlarged perithecia. $\times 60$. E, Primordium of perithecium. $\times 450$. F, Immature perithecium, showing character of contents and early development of perithecial wall. $\times 450$. G, Cross section of perithecia, indicating their position in relation to host tissues. $\times 70$. H, Cross section of a mature perithecium. $\times 450$. I, Representative ascus, showing typical shape and thickened apex of wall. $\times 960$. J, Typical shape of spores. $\times 1,125$. K, Germinating spores. $\times 770$

throw them 3 to 5 mm. Although the development of the perithecia begins within the tissues of the green leaves shortly after the appearance of the conidial stage, the asci do not ordinarily mature before late winter or early spring. Attempts to hasten the maturity of the perithecia and the formation of the ascospores by placing the infected leaves in a moist chamber in the laboratory have proved unsuccessful, except in one case when mature asci were produced in the laboratory in January.

The asci are hyaline, cylindrical clavate, and are not accompanied by paraphyses. (Fig. 3, G and I.) The ascospores are hyaline and 1-septate, each spore being constricted into two slightly unequal cells. The cell which is pointed toward the base of the ascus has a blunt end; the other cell is thickest at a point near the septum and then tapers gradually. (Fig. 3, J.) The method by which the spores are liberated from the asci is not definitely known. The fact that spores may be collected by inverting a plate of agar over leaf tissues containing perithecia indicates that they are ejected through the ostiole with some force.

The relationship of the conidial and the perithecial stage is shown by their constant association, by the development of the conidiophores from hyphae forming part of the perithecial wall, and by the production of similar conidia in artificial cultures from monospore cultures of ascospores. The genetic relation of the pycnidia to the other two spore forms is shown by their regular appearance on the same lesions with the conidiophores and perithecia and by their formation in cultures made from either conidia or ascospores.

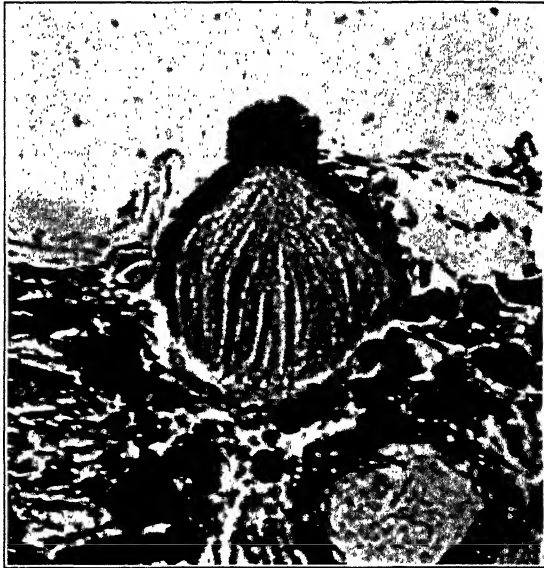


FIGURE 4.—Photomicrograph of an immature perithecium of *Mycosphaerella dendroides*. $\times 1,500$

IDENTITY OF THE ORGANISM

In 1890 B. D. Halsted collected a fungus on hickory leaves near Cold Spring, N. Y. Later Ellis and Everhart (3) described this fungus as *Cercospora halstedii*. The writers have not been able to find any other reference in literature to this fungus except one made by Fawcett (4) in 1909 and one by Waite (10, p. 188) in 1911. Fawcett referred to a disease of pecan leaves in Florida which "produces large brown or black blotches from one-fourth to three-fourths inch in size and irregular in outline," but he did not describe the causal fungus. From the meager discussion one can not be certain

whether Fawcett was dealing with *C. halstedii* or with *C. fusca* Rand, a widely distributed fungus which causes circular to irregular brown spots on pecan leaves. *C. fusca* was first described by Heald and Wolf (5) in 1911 as *Clasterosporium diffusum*, but the name was later changed by Rand (7) to *Cercospora fusca*.

On several occasions the writers have collected near Thomasville, Ga., on hickory leaves, a fungus which is considered by W. W. Diehl as *Cercospora halstedii*. In all essentials the conidial stage of the fungus causing the pecan-leaf blotch reported in this paper is the same as *C. halstedii* found on hickories in Georgia, and the two forms are considered by the writers as identical. While both conidia and pycnosporos taken from hickory and pecan leaves have been compared, mature perithecia have not been observed on hickory specimens collected in southern Georgia.

In 1911 Rand (6) reported a leaf-blotch disease of the pecan (which he later called anthracnose) and described it as *Mycosphaerella converula* (Schw.) comb. nov. Apparently no specimen of his *Mycosphaerella* was preserved, and a comparison can not be made. Rand continued his studies of this pecan-leaf fungus and reported two years later (7, p. 319) that "further cultural studies of the fungus brought out changes in its morphology sufficient to throw it out of the genus *Mycosphaerella*." He summarized his reasons for changing the determination of the fungus as follows (7, p. 329):

From the general pathology and temperature relations, the cross-inoculations and cultural studies, and finally from the morphology of the pecan fungus there can be no doubt of its specific connection with *Glomerella cingulata* (Stonem.) S. and v. S., the fungus causing bitter-rot of apples, ripe-rot of grapes, and anthracnoses of a wide range of hosts.

The outstanding facts brought out in that paper which seemed to have influenced Rand to reclassify the fungus were that the ascospores gradually changed in cultures from a 1-septate to a unicellular form, that the diseased areas on the leaves and cultures regularly produced a *Gloeosporium* type of conidia, and that the fungus when inoculated in apples produced a decay typical of apple bitter rot.

Rand's later report seems to preclude any reason for considering the *Mycosphaerella* reported in his earlier publication as being related to the one described in this paper. In 1927 the writers⁴ provisionally referred the pecan leaf-blotch fungus to *Mycosphaerella* (*Sphaeria*) *converula* Schw., but after making a more critical study of the type specimen of that fungus they concluded that, on account of morphological differences between the two fungi, the reference was not tenable.

In 1822 Schweinitz (8) described a fungus found on *Quercus* as *Sphaeria dendroides*. Later, however, he published a note (9) which indicated that he regarded *S. dendroides* as a synonym of *S. myriadea* Fr. Seemingly the next reference to this specific name in literature was made by Cooke (2, p. 108), who described a fungus found on *Carya* as *Sphaerella dendroides* Schw. Cooke mentioned that "this species has been confounded with *Sphaeria myriadea* from which it is evidently distinct." Cooke's description is apparently based on specimens issued by Ravenel in his *Fungi Caroliniani* as No. 61.

⁴ DEMAREE, J. B., and COLE, J. R. TWO UNREPORTED LEAF SPOTS OF PECANS. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 11: 135-136. 1927. [Mimeographed.]

The specific name that he used was that of Schweinitz in a new combination, the original name having been applied by Schweinitz to a fungus on oak, doubtless correctly referred to as *Sphaeria myriadea* Fr. It seems, therefore, that the name *Sphaerella dendroides* is really not that of Schweinitz, but should be quoted as *S. dendroides* Cke.

Morphologically, the perithecial stage of the pecan leaf blotch and *Sphaerella dendroides* as described by Cooke (2) appear to be identical. The size and shape of the ascospores correspond quite closely. Cooke stated in his description that the ascospores of *S. dendroides* were 4μ wide and 23 to 24μ long. Since the name *Mycosphaerella* is in better usage than *Sphaerella* and is now employed to designate fungi forming perithecia and spores resembling those described in this paper, the writers suggest that the pecan leaf-blotch fungus be regarded as *Mycosphaerella dendroides* (Cke.) comb. nov.

EMENDED DESCRIPTION

Mycosphaerella dendroides (Cke.) comb. nov. *Sphaerella dendroides* Cke. 2, p. 108), not *Sphaeria dendroides* Schw. (8, p. 47).

Perithecia hypophyllous at first, subepidermal, finally erumpent, single or crowded, aggregate in irregular groups, or more or less over the entire lower leaf surface; globose to irregularly subglobose, 75 to 125μ in diameter, shiny black, later roughened. Perithecial walls usually four to six cells thick, thicker around ostiole. Asci clavate to cylindrical, thin walled, thickened at apex, hyaline, 38 to 66μ by 10 to 15μ . Ascospores 18 to 26μ by 4 to 6μ .

Pycnidia erumpent, black, globose to spherical, conical, 75 to 90μ wide. Pycnospores hyaline, nonseptate, 2 to 4μ by 0.5 to 1μ .

Conidia subhyaline to light brown, elongate, subclavate, rostrate, terminal or lateral, solitary or in chains of two to three, 35 to 83μ by 4.5 to 7μ , 1-septate to 8-septate, usually with a conspicuous vacuole within each segment. Apical segment long and attenuate. Conidiophores erect to prostrate, simple or branched, light to dark brown, emerging either singly or in groups of two to eight. Hyphae intercellular within the tissue of mature leaves, subhyaline, branched, and septate.

The fungus is parasitic in mature leaves of *Hicoria* species. The perithecia first appear on green leaves during the latter part of the growing season and mature on fallen leaves the following spring. The perithecial stage has also been reported on leaves of *Hicoria* from South Carolina.

PHYSIOLOGY

GROWTH ON ARTIFICIAL MEDIA

Some difficulty was at first experienced in obtaining cultures from either conidia or ascospores. Only about 10 per cent of the conidia and a smaller percentage of the ascospores have been induced to germinate in the laboratory. The early growth is very slow, the cultures increasing about 50μ in diameter a day for the first 10 days or 2 weeks when grown on corn-meal agar and Lima-bean agar.

Single-spore cultures of the conidial stage of the fungus were obtained in the usual way. Ascospores used for culture work were obtained by catching spores in a plate of agar inverted about 2 to 3 mm. above sections of leaves bearing mature ascospores and lying on wet filter paper. Germination and growth were followed under the microscope, and isolated germinating spores were marked and transferred to another plate of agar. When the cultures became macroscopic in size they were transferred to agar slants.

Monospore cultures require a period of 10 to 12 days under optimum temperature conditions to produce macroscopic growth. The media

used were of three types—nutrient agars, vegetable plugs, and nutrient liquids. Of the nutrient agars, the fungus grew best on those made from Lima beans or dextrose, attaining a maximum growth of 10 mm. in diameter in 60 days. In these two agars the fungus formed a black stroma, slightly crinkled, mostly submerged, and covered with an abundance of white mycelium. On prune agar the fungus formed a black stroma, raised and crinkled, but produced no aerial mycelium. Of the vegetable plugs used, the greatest growth was on sweetpotato. On this medium the stroma was crinkled, elevated, black, and covered with short, grayish mycelium. In the liquid, i. e., corn-meal and nutrient solution, the growth was slight and consisted of a "floating stroma" about 8 mm. in diameter, black and covered with short hyphae.

Conidia are commonly produced in young cultures growing upon media rich in carbohydrates and appear to arise from vegetative hyphae rather than from conidiophores, which are not produced in artificial cultures. The conidia are slightly abnormal and do not always have the regular shape and the same number of septa as those found on the host.

Pycnidia containing the characteristic small rod-shaped pycnospores found on pecan leaves are produced in abundance in both old and new cultures. Although cultures of this fungus have been under observation for three years, no evidences of the presence of perithecia have been noted.

TEMPERATURE AND PH RELATIONS

The temperature for the growth of this organism ranges from 16° to 31° C. The optimum is 27° and the lethal is about 36°. The fungus makes little or no growth when subjected to a temperature of 16° or below. In the studies of the fungus with respect to hydrogen-ion concentration, corn-meal agar with a pH reading ranging from 3.5 to 9.7 was used. The cultures were held at the optimum temperature (27°) for 60 days before the final reading was made. Sparse growth occurred at pH 3.5 to 5.2 without production of aerial mycelium. The maximum growth was made at pH 7.5. The color of the aerial mycelium on the stroma in the hydrogen series ranged from olive green to dark brown.

PATHOGENICITY

The exact conditions that favor germination and infection have not been demonstrated. Infections have never been known to occur on young pecan leaves and have been found on full-grown ones only after they have assumed the dark-green color. The writers do not know whether this indicates that young leaves are immune or that the incubation period extends over a relatively long period. Repeated attempts to infect the leaves of potted seedlings in the laboratory and the leaves of trees in orchards and nurseries by means of conidia and ascospores have been unsuccessful. When the work was done in the laboratory the inoculated plants were covered with a bell jar for 24 to 48 hours, and the inoculated leaves of larger trees outdoors were inclosed in either glassine bags or celluloid chambers for 24 hours.

Since artificial inoculations with both conidia and ascospores have not resulted in infection, it has not been possible to comply with Koch's rules; but the production of conidiophores and conidia on

living leaves in areas at first nonchlorotic shows beyond doubt the connection of the conidial stage of the fungus with the disease. The presence of the fungus itself is one of the first signs of the disease.

CONTROL

Orchard and nursery sanitation will undoubtedly play an important part in the control of the pecan leaf blotch. Cultural practices for effecting orchard sanitation, now coming into general use in the control of pecan scab, will undoubtedly have to be extended as aids in the control of other pecan diseases. The leaf-blotch fungus is not known to attack any part of the pecan tree except the leaf blades. As the fungus overwinters only on these parts, it is reasonable to assume that if the leaves are plowed under during late winter or early spring infection of the new crop of leaves will be materially lessened.

The writers have observed that blotch is almost absent in orchards where spray or dust was used against pecan scab. Three applications of Bordeaux mixture or four or five applications of monohydrated copper sulphate and lime dust have uniformly reduced blotch to a negligible factor.

During the season of 1928 a preliminary experiment was made to test the efficacy of a dust composed of 20 per cent monohydrated copper sulphate and 80 per cent hydrated lime as a control for pecan leaf blotch in nurseries. The work was done near Cairo, Ga., in a block of 3-year-old nursery trees, which was divided into five plots and treated on the following dates: Plot 1, May 11 and 31, June 22, July 16 and 30; plot 2, May 31, June 22, July 16 and 30; plot 3, June 22, July 16 and 30; plot 4, July 16 and 30; and plot 5, July 30.

The control of the disease in plots 1, 2, and 3 was excellent, and only a slight difference could be noticed among them. Considerable blotch was present, however, in plots 4 and 5. A comparison of A and B of Figure 5 shows clearly what was accomplished with five applications of dust.

The results of the test brought out two important facts. They showed that under the conditions of the experiment the disease was readily controlled with the copper-lime dust and they indicated that the June and July applications were the most important and that earlier treatments may not be necessary to effect commercial control.

SUMMARY

Leaf blotch, a disease of pecan leaves caused by the fungus *Mycosphaerella dendroides* (Cke.) comb. nov., is known to occur in Georgia, Florida, Alabama, Mississippi, Louisiana, and Indiana. The disease is rapidly increasing in importance and seems to be extending its range of distribution. It is also known to occur in South Carolina and Georgia on other species of *Hicoria*.

The conidial stage of the fungus which attacks mature leaves of the host during the latter half of the season is here considered identical with *Cercospora halstedii* E. and E. The fungus is different from *C. fusca*, a widely distributed pecan parasite.

The most conspicuous sign of the disease is the presence of groups and blotches of pycnidia and immature perithecia on the undersides

of the leaf blades. The asci mature on the fallen leaves during the spring.

The morphological characters of the conidial, pycnidial, and ascogenous stages are described.

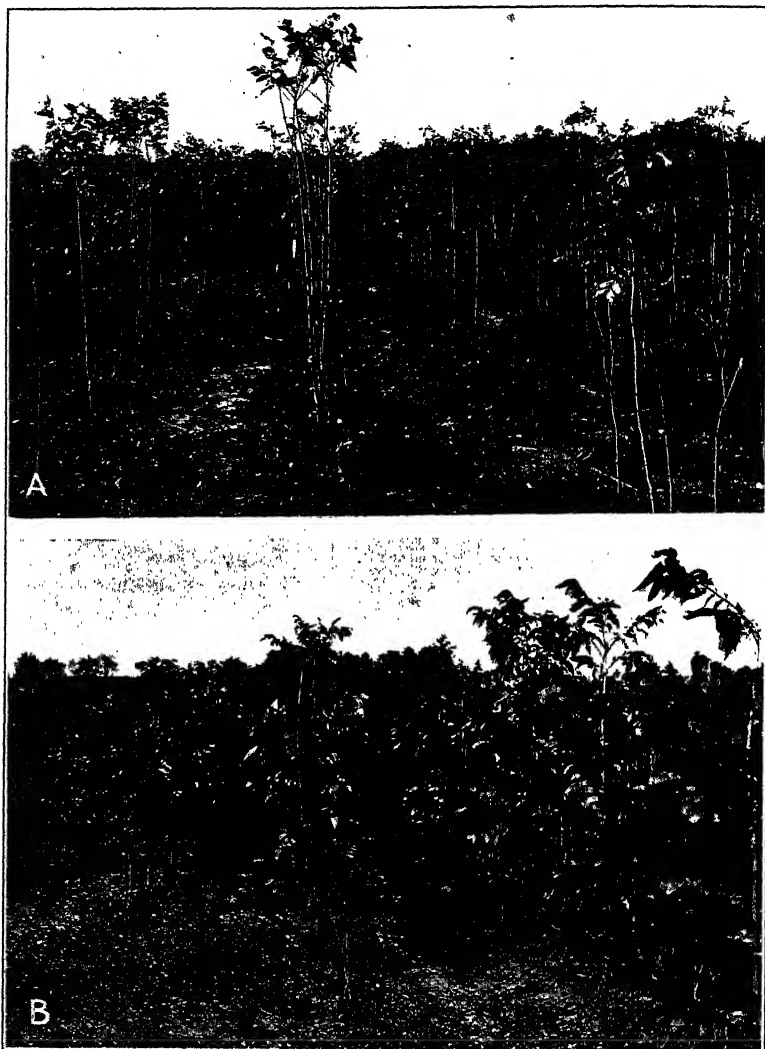


FIGURE 5.—A, Untreated plot, showing defoliation of nursery pecan trees by the leaf-blotch fungus; B, plot dusted five times with a mixture of 20 per cent monohydrated copper sulphate and 80 per cent hydrated lime. Both plots photographed November 16, 1928

The relationship of conidial and perithecial stages is indicated by both stages being found on the same lesions, by the similarity of cultures from either conidia or ascospores, and by the production of similar conidia and pycnidia in artificial cultures made from either conidia or ascospores.

Orchard sanitation will undoubtedly play an important part in the control of the disease. A preliminary experiment indicated that monohydrated copper sulphate and lime dust will effectively control the disease and that midsummer treatments are the most important.

LITERATURE CITED

- (1) BUBAK, F.
1906. EINIGE NEUE PILZE AUS NORD AMERICA. Jour. Mycol. 12: 52-56.
- (2) COOKE, M. C.
1883. ON SPHAERELLA AND ITS ALLIES. Jour. Bot. [London] 21: 67-71,
106-110, 136-139.
- (3) ELLIS, J. B., and EVERHART, B. M.
1892. NEW SPECIES OF FUNGI FROM VARIOUS LOCALITIES. Acad. Nat. Sci.
Phila. Proc. 1891: 76-93.
- (4) FAWCETT, H. S.
1909. REPORT OF PLANT PATHOLOGIST. Fla. Agr. Expt. Sta. Ann. Rpt.
1909: xlvii-lxii, illus.
- (5) HEALD, F. D., and WOLF, F. A.
1911. NEW SPECIES OF TEXAS FUNGI. Mycologia 3: 5-22.
- (6) RAND, F. V.
1911. A PECAN LEAF-BLOTCH. Phytopathology 1: 133-138, illus.
- (7) ———
1914. SOME DISEASES OF PECANS. Jour. Agr. Research 1: 303-338, illus.
- (8) SCHWEINITZ, L. D. DE
1822. SYNOPSIS FUNGORUM CAROLINAE SUPERIORIS ... Ed. by D. F.
Schwaegrichen. 105 p., illus.
- (9) ———
1834. SYNOPSIS FUNGORUM IN AMERICA BOREALI MEDIA DEGENETIUM.
Amer. Phil. Soc. Trans. 4: 141-316.
- (10) WAITE, M. B.
1911. NUT DISEASES; WITH SPECIAL REFERENCE TO THE PECAN. Amer.
Pomol. Soc. Proc. 32: 182-190.

CELL-SAP CONCENTRATION AND TRANSPIRATION AS RELATED TO AGE AND DEVELOPMENT OF COTTON LEAVES¹

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INTRODUCTION

This paper deals with the interrelations between the concentration of cell sap and the rate of transpiration of cotton leaves as influenced by age, and the relation of these factors to leaf development.

The experimental plants were of the Pima variety of Egyptian cotton grown as a part of a water-requirement series at Sacaton, Ariz., in 1927. During the growth of the plants all lateral buds were removed as they appeared. Limiting aerial growth to the main axes and leaves resulted in vigorous plants provided with an unusual number of leaves on the main stalk. The date of appearance of each of the leaves was recorded throughout the summer.

No procedure for the direct determination of the individual transpiration rates of attached leaves fully exposed to the weather has yet been devised. It is possible, however, to obtain relative values by the leaf-temperature method, as measurements already reported (5)³ have shown that the differences in the temperatures of similarly exposed cotton leaves are inversely proportional to the differences in their transpiration rates. The correlation found between the hourly differences in the temperatures and transpiration rates of the leaves of plants in dry and moist soils was -0.93 ± 0.025 . This inverse relationship between transpiration and leaf temperature is graphically illustrated in Figure 1.

Weighing is the most direct method of studying transpiration, and where whole plants are used it yields the most reliable information. Many investigators, however, have used the method of comparing the transpiration rates of cut leaves. Deductions from such measurements are of uncertain value, since it can not be assumed that they are applicable to attached leaves on rooted plants. Palladin (15, p. 138) cited Krutizky to the effect "that a single leaf of *Cyssus antarcticus* lost 10.6 c. c. of water in one day, while a branch of the same plant with six leaves lost but 10.8 c. c." Hygrometric paper has been used in studying transpiration, and the method has certain advantages over direct measurements in that it can be used on attached leaves. The hygrometric-paper method is tedious and requires careful comparisons with standard colors, in which comparisons time and temperature are both factors of some significance. The great disadvantage of this method is that it does not measure the normal transpiration rate of a leaf but gives only an index to what is termed the "transpiring power."

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² The photographs for Figures 2, 3, and 5 were taken by H. F. Loomis, Assistant Agronomist, Office of Cotton, Rubber, and Other Tropical Plants.

³ Reference is made by number (italic) to "Literature cited," p. 802.

The portion of the leaf studied is covered with the paper and a glass slide during the observation.

The leaf-temperature method is not only convenient and applicable to attached leaves, but also it has a further advantage in that it is rapid. The leaves, until the moment of a measurement, are fully exposed to the weather, and during the few seconds required for a reading by the procedure employed there is little opportunity for a material change in leaf temperature. The high correlation found between transpiration rates and leaf temperatures suggested the

possibility of using the method to secure the measurements and comparisons here reported.

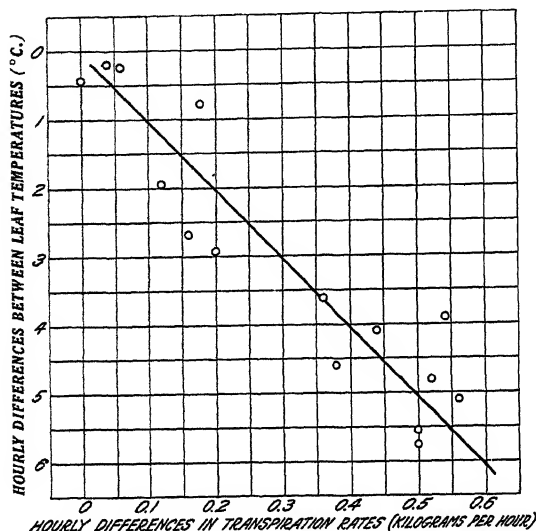


FIGURE 1.—Relation between hourly leaf-temperature differences (wilted minus turgid) and the corresponding hourly differences in transpiration rates (turgid minus wilted) of cotton plants grown in dry and in moist soils. The transpiration values are each for a full hour, whereas the leaf temperatures were measured over a much shorter period at the middle of the hour. Fluctuations in environmental factors quickly influence leaf temperatures as they do transpiration rates, but such fluctuations may make only a minor impression on the total transpiration of an hour. These measurements, which cover one day, have been shown (\bar{x}) as an hour graph of the two variables with respect to time.

REVIEW OF LITERATURE

An examination of the literature dealing with the sap concentration and the transpiration rate of leaves of different ages brings out the fact that investigators, using various methods, have found great variability in the directions that these gradients take in different plants.

Schechner⁴ reported that the transpiration of young leaves of many plants was greater than that of older leaves and in some plants leaves of

intermediate age had lower transpiration rates than either older or younger ones. Fleischmann and Hirzel,⁴ on the contrary, found equal surfaces of old hop leaves to transpire 2.5 times as much water as young leaves. Seeliger,⁴ working with cut leaves from woody plants, drew conclusions somewhat similar to those of Schechner, as did Overton (14) working with *Cyperus papyrus*. Pringsheim (16), working with succulents, stated that for equal weights old leaves of *Sedum spectabile* lost more water than young leaves, whereas with *Sempervivum braunii* the relation was reversed. Giddings (8) found that both the higher and lower leaves of *Silphium laciniatum* transpired less rapidly than leaves borne near the middle of the plant. Koketsu (11), using standardized hygrometric paper, found the stomatal transpiring power of Coleus leaves to be greatest at the height of their development. Palladin (15, p. 139) has generalized by saying:

⁴ Cited by Burgerstein (1).

During the period of greatest activity of the leaf, while it is still growing, the rate of transpiration is highest. The reason for this is that the epidermis of young leaves is very permeable to water; transpiration decreases later, but a second maximum is reached when the stomata begin to function. Thereafter the rate of transpiration gradually decreases as the epidermis hardens, in spite of the influence of the stomata.

Pringsheim (16) reported the sap concentrations of the old leaves of the two succulents mentioned to be lower than those of the young leaves. Dixon and Atkins (3), working with lilacs and other plants, and Chandler (2), working with apples and peaches, found the older leaves to have higher sap concentrations. Reed (17), however, could find no real difference in the sap concentrations of leaves at opposite ends of new apricot shoots. Hurd-Karrer (10) stated that the specific gravity of the sap expressed from the internodes of cornstalks was higher toward the top of the plant. Fernald (6) notices a tendency, with irregularities, for the sap of leaves of *Philadelphus* and privet to increase in concentration toward the tips of branches. In two cases a marked drop in the sap concentrations of the uppermost leaves was shown.



FIGURE 2.—Thermocouple mounting and method of clamping a leaf preparatory to a leaf-temperature reading. The air junction is protected from the direct sunlight by the paper umbrella

METHODS

The temperatures of the leaves were measured in terms of their departures from the temperature of the air. A wall galvanometer, mounted on a screened porch some 40 feet from the experimental plants, was connected by flexible lighting cord to the thermocouples. These were inclosed, except for the junctions, in a piece of semi-flexible composition tubing conveniently attached to a wooden

handle. (Fig. 2.) With one of the thermocouple junctions protected from the direct rays of the sun by two tightly separated slips of white paper, the second junction was brought firmly into contact with two portions of an upper leaf surface by folding the leaf upward with a



FIGURE 3.—Pima cotton plants grown without branches or bolls. The tall plants were used for the measurements reported in this paper. The leaves of the short plants, which had lost their terminal buds through worm injury, became greatly enlarged and thickened and were very brittle. The sap of the three upper leaves of the short plant had a freezing-point depression approximately 15 per cent greater than that of the leaves of corresponding age from the tall plants. Sacaton, Ariz., September 2, 1927

pair of cork-tipped crucible tongs. As each leaf was thus secured the galvanometer deflection was recorded by an observer seated on the porch. A more detailed description of the method as well as a discussion of this and other methods of measuring leaf temperatures is given in the paper cited (5).

Four temperature measurements were made on each of the leaves of two plants. (Fig. 3.) Beginning with the lowest leaf that did not show marked signs of deterioration, the temperature of each leaf was measured in turn up the main stalk without regard to the exposure of the leaves to the sun. As soon as the temperatures of all the leaves on one of the plants had been secured the pot was given a quarter turn to bring a new set of leaves into the more direct light and the measurements were then started on the plant in the second pot. This procedure was repeated four times. Immediately following the com-

pletion of the temperature measurements all the leaves were gathered for determination of the cell-sap concentration. Each of the sap samples was made up of six leaves, three from each plant for each age group. The mean temperatures of the leaves in each of these groups were averaged for the comparisons. The above-described procedure

provided 24 readings for each temperature mean and six leaves for each sap sample, except that two leaves from one of the plants and one from the other were missing or mutilated. The temperature of the uppermost leaf on one of the plants and the two uppermost leaves on the other (Nos. 45 and 46 in Table 1) were also measured, but these leaves were so small (3 and 4 cm. long) that it was not feasible to collect them for the leaf-sap measurements. Leaves 42 to 44 did not yield sufficient sap for a conductivity determination.

TABLE 1.—*Ages, temperature differences, and freezing-point depressions and conductivities of the expressed sap of leaves at successively higher nodes on the main stalks of two Pima cotton plants grown without branches, at Sacaton, Ariz., September 2, 1927*

Leaves Nos.	Mean age of 6 leaves	Difference between leaf and air temperatures	Freezing-point depression (Δ.) of sap	Specific electrical conductance (κ) at 30° C.
	Days	° C.	° C.	Reciprocal ohms
12-13-14.....	82	-2.3±0.23	1.52	0.0316
15-16-17.....	74	-2.7±.27	1.57	.0329
18-19-20.....	68	-2.5±.20	1.56	.0333
21-22-23.....	62	-2.8±.30	1.55	.0337
24-25-26.....	54	-3.1±.24	1.47	.0325
27-28-29.....	46	-3.1±.20	1.44	.0319
30-31-32.....	39	-3.5±.16	1.45	.0312
33-34-35.....	32	-4.0±.16	1.39	.0295
36-37-38.....	26	-4.1±.11	1.39	.0295
39-40-41.....	20	-4.1±.15	1.33	.0288
42-43-44.....	10	-3.7±.13	1.10
45-46.....	3	-2.5±.23

As the concentrations of the cell sap were measured by the methods used extensively by J. A. Harris and his collaborators (e. g., Gortner and Harris, 7), it is not necessary to describe them in detail. At the time of collection the leaves were pressed into tubes and then frozen in a salt-ice mixture for 24 hours. The sap was then expressed from the leaves and the freezing-point depressions and conductivities were determined as soon as possible, the various samples being kept cold until ready for use.

EXPERIMENTAL DATA

The leaf-temperature measurements were made and the leaves were collected for the expressed-sap determinations on September 2, 1927. The temperature measurements were started at 2.17 and ended at 2.47 p. m. A light breeze was blowing while the measurements were being made and the sky was apparently clear, although the sunshine recorder showed a slight but irregular haze insufficient to lower materially the insolation. The air temperature at 2.15 p. m. was 40° C. and the wet bulb was 21°, the relative humidity therefore being 17 per cent. The results of the measurements are presented in Table 1 and shown graphically in Figure 4.

The leaves are grouped in Table 1 according to both age and position on the plant. The ages designated are the means of the leaves in each group. The two plants developed new leaves at very nearly

the same rate, and the corresponding groups of three leaves on the two plants rarely differed in mean age by more than two days. One of the plants was 192 and the other 188 cm. tall on the date of the measurements.

LEAF TEMPERATURE

The leaves (fig. 4) were found to be progressively cooler from the older upward toward the younger until leaves but 10 days old were reached. The oldest leaves had the highest temperatures, 2.3° C. below air temperature. Leaves 26 and 20 days old were the coolest, 4.1° below air temperature. Leaves 10 days and 3 days old were

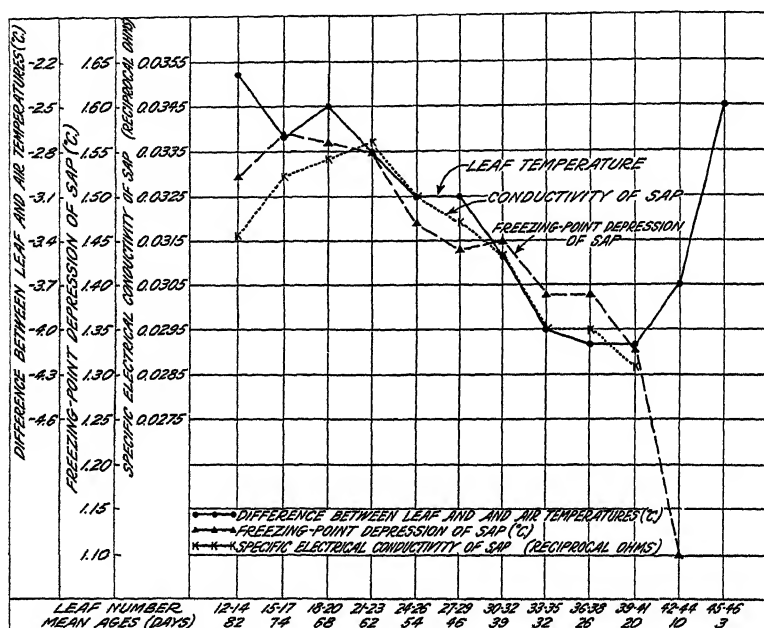


FIGURE 4.—Ages, temperature measurements, and freezing-point depressions and conductivities of the expressed sap of leaves at successively higher nodes on the main stalks of two Pima cotton plants grown without branches, at Sacaton, Ariz., September 2, 1927

warmer than the 20-day-old leaves and had temperatures of 3.7° and 2.5° , respectively, below that of the air.

The probable errors of the means (Table 1) indicate that between successive age groups the temperature differences in general lack statistical significance. It is to be observed, nevertheless, that the temperature curve is both definite in its course and relatively smooth. The variability of the temperatures within each group is to be attributed to the fact that in each group during each of the four series of measurements there were always a number of shaded leaves. Since in each age group there was approximately the same number of shaded or partially exposed leaves, the means doubtless possess a greater significance than that indicated by the probable errors.

OSMOTIC CONCENTRATION

The freezing-point depressions of the expressed sap of the leaves also yielded a curve which is quite definite in character. The osmotic concentrations decrease with the age of the leaves and the height of their insertion. The marked decline in sap concentrations between leaves 20 and 10 days old is noteworthy and suggests that the concentrations in the next younger group, 3-day-old leaves, may have been very low. Between leaves 20 and 10 days old there is a difference of 2.8 atmospheres in the osmotic concentration of the sap.

SPECIFIC ELECTRICAL CONDUCTIVITY

The sap of the leaves of the 62-day group had the highest conductivities, the values for both the older and the younger leaves being lower. The freezing-point depressions indicate a depletion of total solutes in the oldest group of leaves only, whereas the conductivity measurements indicate a depletion of electrolytes in the three oldest groups.

Between the leaves 62 and 20 days old the freezing-point depressions decreased by 14.2 per cent and the conductivities decreased 14.5 per cent. This proportional agreement in change suggested either that the electrolytes and nonelectrolytes decreased in the same proportion or that the nonelectrolytes were present in quantities too small to contribute materially to the freezing-point depressions.

For the purpose of determining which of the possibilities just mentioned was the more plausible, the following computations were made: If it is assumed that the conductivity of these samples was uninfluenced by the presence of organic solutes and colloids it is possible to compute roughly the relative proportions of electrolytes and nonelectrolytes in the samples. By applying the factor 30^5 to each of the conductivity values, results are obtained which represent what theoretically should be the approximate freezing points for the electrolytes alone. Subtracting the products so obtained from the corresponding observed freezing points, the remainders should equal the freezing points of the nonelectrolytes. The latter values for these samples range from 0.47° to 0.58° C. These values, however, are badly out of line with the knowledge of the sugar content of cotton leaves. The amount of sugar typically found in cotton leaves, if all dissolved in the plant sap, would in itself yield a freezing point in the order of but 0.05° to 0.15° C. It must therefore be concluded that conductivity values obtained from plant saps are not closely indicative of the abundance of electrolytes.

Mason (13) found that the addition of known quantities of potassium chloride to plant sap did not result in the expected increase in conductance. This he attributed to the viscosity of the sap and attempted to make corrections on that basis. The influence of an organic solute on the conductivity of potassium chloride solutions is illustrated in Table 2.

⁵ The factor 30 assumes that sulphates and chlorides were present in the relative proportions found by Harris, Hoffman, and Lawrence (9) in Pima cotton leaves at Sacaton and that the predominating cation was sodium.

TABLE 2.—*Specific electrical conductance of 0.1 N potassium-chloride solutions containing increasing quantities of sucrose*

Sucrose concentration per liter of solution (gm.)	Percentage of volume occupied by sucrose	Specific electrical conductance (κ) at 20° C.	Ratio of conductance of solution with sucrose to conductance of solution without sucrose
0	0	0.0117	1.00
79	5	.0103	.88
158	10	.0085	.73
237	15	.0070	.60

Table 2 shows the conductance of a solution containing 0.7 mole of sucrose and 0.1 mole of potassium chloride to be 40 per cent less than that of a pure solution containing the same amount of potassium chloride per liter of solution. For each 1 per cent of the volume occupied by the sucrose the specific electrical conductance was decreased by about 2.5 per cent.

While in no way detracting from the biological significance to be attached to the comparative osmotic concentration of leaf saps, the conclusion is inevitable that neither freezing-point depression nor conductivity values either singly or together furnish reliable information as to the proportion of a freezing point attributable to organic solutes, nor do conductivity measurements on plant sap afford more than an indication with respect to the quantity of electrolytes present.

CORRELATION BETWEEN LEAF TEMPERATURE AND CELL-SAP CONCENTRATION

Values for the leaf-temperature departures and for the freezing points and conductivities of the expressed sap were each obtained for the leaves in age groups from 82 to 20 days. The leaf temperatures and freezing-point values for these groups are found to be correlated to the extent of -0.92 ± 0.114 . The coefficient of correlation between leaf temperature and conductivity is -0.86 ± 0.061 . Both correlation coefficients are high and indicate a close inverse relationship between the changes in leaf temperature and in the concentration of the expressed sap. In view of the previously cited high correlation between leaf-temperature and transpiration differences, the inference appears justified that there is a close inverse relationship between the changes in sap concentration and the rate of transpiration, i. e., low sap concentrations are conducive to high transpiration rates and reduced leaf temperatures. An agreement even closer is evident in the age groups from 62 to 20 days. This latter series of leaves was looked upon as being neither senescent with regard to cell deterioration nor immature with respect to epidermal characteristics and the development of stomata and intercellular spaces.

While it is possible that the high coefficient of correlation found between leaf temperature and sap concentration may not indicate a causal relationship, there is other evidence which suggests that the rate of transpiration may be intimately associated with the concentration of sap. In previous work (4) with Australian salt-

bush and wheat, for example, it was found that when these plants were grown on soils with added sodium chloride there was an increase in the sap concentration with each increment in the salt content of the soil. The sap concentration of the saltbush was increased 42 per cent by this method, and this increase was accompanied by a 44 per cent decrease in the water requirement. Wheat grown on the same soils showed an increase of 72 per cent in the sap concentration and a decrease of 69 per cent in the water requirement between soils with 0.05 and 0.25 per cent of added sodium chloride, but it had a lower water requirement on the control soil than on the 0.05. It has also been found (5) that cotton watered with a very saline water had a higher sap concentration and a lower water requirement than when water containing approximately one-fourth as much salt was used. Cotton grown on soils with added sodium chloride has not shown consistent results.



FIGURE 5.—Upper portions of six Pima cotton plants grown without branches or bolls. The two center plants were defoliated on September 2, 1927. The leaves produced after defoliation were lighter green, of finer texture, and much larger than the leaves formed on the control plants during the same period. Sacaton, Ariz., September 25, 1927

LEAF DEVELOPMENT FOLLOWING DEFOLIATION

It has been noted, particularly perhaps in arid regions, that after trees or other plants have been heavily pruned the new leaves that develop are frequently larger and of a more mesophytic character than those cut away. While such reactions may be looked upon as an indication that pruning in some manner exerts an invigorating effect upon the plant, the measurements just reported suggest an alternative explanation on the basis of water relations. As may be observed in Figure 5, the new leaves produced by the cotton plants after the leaves had been removed for sap determinations were quite different from leaves produced during the same period by otherwise similarly treated plants from which the leaves had not been removed.

The upper portions of the two Pima cotton plants are shown as they appeared 23 days after defoliation. The new leaves which appeared on the defoliated plants were of a softer texture, very

much larger, thinner, and lighter green than those on the control plants shown at either side. The comparative elongation rates and the rate of new leaf development are given in Table 3.

TABLE 3.—Comparison between 2 defoliated and 15 control Pima cotton plants, with respect to elongation rates and the unfolding of new leaves, before and after September 2, when defoliation took place

MEAN DAILY ELONGATION OF PLANTS (CM.)

Description of plants	Before defoliation				After defoliation				
	Aug. 8-14	Aug. 15-21	Aug. 22-28	Aug. 29- Sept. 1	Sept. 2-4	Sept. 5-11	Sept. 12-18	Sept. 19-25	Sept. 26- Oct. 9
Control.....	2.4	1.6	1.7	1.3	1.6	1.6	1.3	1.4	0.6
Defoliated.....	2.6	1.5	1.5	1.3	1.0	1.0	1.0	1.5	.6

MEAN DAILY NEW LEAF FORMATION PER PLANT (NUMBER)

Description of plants	Aug. 8-14	Aug. 15-21	Aug. 22-28	Aug. 29- Sept. 1	Sept. 2-4	Sept. 5-11	Sept. 12-18	Sept. 19-25	Sept. 26- Oct. 9
	0.46	0.31	0.23	0.38	0.27	0.46	0.34	0.23	0.10
Control.....	.46	.31	.23	.38	.27	.46	.34	.23	.10
Defoliated.....	.43	.29	.29	.38	.50	.57	.43	.29	.11

The height of plant measurements show that the elongation rates of the defoliated plants were less than those of the control plants for approximately 10 days immediately following defoliation, after which period it appears that the defoliated plants may have grown slightly faster than the controls. Defoliation did not retard, even temporarily, the formation of new leaves. Between August 8 and September 2, before defoliation, there were unfolded on both the experimental and the control plants a mean of 8.5 new leaves per plant, whereas between September 2 and October 10, after defoliation, the control plants produced 9.5 leaves per plant and the defoliated plants produced 12 leaves each. Comparative measurements of the areas of the leaves on the control and defoliated plants were not secured, but it was estimated that the new leaves on the defoliated plants attained an area not less than three times that of the leaves of the same age on the control plants.

The availability of considerable quantities of reserve translocatable materials may be looked upon as prerequisite to an increased leaf expansion as marked as that shown by these plants. The complete defoliation of plants without such reserves would doubtless have been followed, at least temporarily, by weak growth if not by death. Ludwig (12), who studied the relation of defoliation to yields of cotton, reported that in irrigated plots many plants died as a result of late-season defoliation. There is some evidence, aside from the reaction itself, which suggests that the writer's group of plants were operating with an abundance of hydrolyzable material in reserve, the presence of which can be most readily attributed to the fact that they were grown without bolls. It is improbable, however, that considerations involving nutritional relationships would be of great value in explaining the reactions of the plants to defoliation, since plants most abundantly supplied with leaves should, with other things equal, be most able to extend their vegetative development

Figure 5 furnishes convincing evidence that the lower leaves of the control plants exerted a marked influence upon the development of the leaves above. It is not difficult to conceive that the old leaves, approaching senescence, may have liberated growth-inhibiting substances which were translocated upward to the younger leaves to depress their growth and prevent them from attaining the luxuriant development which is so marked in the case of the leaves formed after defoliation on the experimental plants. A hormone theory in this case, however, would require an assumption as to both the presence and the method of action of an unknown chemical substance and therefore must be of uncertain applicability.

The freezing-point depression determinations showed that the sap in the upper leaves of these plants was less concentrated than that in the lower leaves. This difference in osmotic concentration would place upper leaves at a disadvantage with respect to older leaves in their ability to withdraw water from the transpiration stream when old leaves were present and not fully turgid. Chandler's tomato experiment (2) shows this to be more than a theoretical consideration. In his experiment he placed the roots of one of a pair of approach-grafted plants in a sugar solution to increase its sap concentration and the roots of the other in distilled water. When the roots of both were exposed to the air the plant from the sugar solution was able to draw water through the graft and remain turgid at the expense of the companion plant, which wilted. In addition to the difference in osmotic concentration, the upper cotton leaves were also at a disadvantage in their water relations as a result of their greater height of insertion, which would impose both a difference in the lift and additional frictional resistance to water movement.

Not only were the upper leaves of the control plants at a disadvantage with respect to their ability to obtain water, but they were also less able to retain it against transpiration. The leaf-temperature measurements showed higher transpiration rates for the younger than for the older leaves. On those days in which the transpiration rates of these plants exceeded the rates of water absorption and conduction the upper leaves always showed the first signs of wilting.

The difference in the water relations of the young and old leaves appears sufficient to account for the difference in the growth characters of the leaves on the two sets of plants as well as of the upper and lower leaves of the control plants. Leaves of plants under mesophytic conditions are almost always larger, thinner, and of finer texture than the leaves of plants in exposed or dry habitats, as is amply illustrated in the literature. The two factors limiting the quantity of water which may be supplied to transpiring leaf surfaces are the rate of water absorption and the rate of its conduction to the leaves. During periods of high transpiration either one or both of these rates may become limiting factors in the amount of water maintained in leaf tissues. When such shortages exist it is natural that a competition for water between leaves should result. The evidence obtained from the cell-sap concentration determinations indicate that older cotton leaves, because of their relatively high sap concentration, were osmotically at an advantage over younger leaves in their ability to withdraw water from the transpiration stream, and also, as indicated by the temperature measurements, the older leaves required less water to meet the demands of transpiration.

The evidence furnishes confirmation of the view that xerophytic leaf characters under conditions of water stringency, internal or external, should be looked upon as being consequences of the environment rather than as fortuitous adaptations which peculiarly fit plants to their environment.

SUMMARY

The sap concentrations and the temperatures of Pima cotton leaves varying in average age from 82 to 3 days were measured. These leaves were at successively higher nodes of the main stalks of plants grown without branches or bolls.

The freezing-point depressions of the expressed sap from the leaves decreased from 1.57° C. for leaves 74 days old to 1.10° for leaves 10 days old.

The specific electrical conductivities of the same saps increased from 0.0316 reciprocal ohms for leaves 82 days old to 0.0337 for leaves 62 days old, and then decreased to 0.0288 for leaves 20 days old.

Young leaves were found to be cooler than old ones. The temperature of leaves 82 days old was 2.3° C. below that of the air, whereas the temperature of leaves 26 and 20 days old was 4.1° below. The temperature of very young leaves, 3 days old, was approximately the same as that of leaves 82 days old. The coefficient of correlation between the temperature and the sap concentration of leaves from 82 to 20 days old was -0.92 ± 0.035 for the freezing point and -0.86 ± 0.061 for conductivity. Reference is made to an earlier paper (5), in which it was shown that the differences in the temperatures of cotton leaves with different transpiration rates were inversely proportional to the transpiration differences.

The plants defoliated for the leaf-sap measurements subsequently developed leaves of a more mesophytic character and of approximately three times greater size than the coincident leaves on similarly treated plants that had not been defoliated. The leaf-temperature and sap-concentration measurements are looked upon as furnishing an explanation of the stimulated leaf development on the basis of differences in the water relations of the new leaves on the two sets of plants. Young leaves of the cotton plants with their low sap concentration were less able than the old leaves to obtain water from the transpiration stream, and their higher rate of transpiration served to increase their requirements per unit of area.

LITERATURE CITED

- (1) BURGERSTEIN, A.
1920. DIE TRANSPIRATION DER PFLANZEN. t. 2, 264 p., illus. Jena.
- (2) CHANDLER, W. H.
1914. SAP STUDIES WITH HORTICULTURAL PLANTS. Missouri Agr. Expt. Sta. Research Bul. 14: 491-552, illus.
- (3) DIXON, H. H., and ATKINS, W. R. G.
1912. CHANGES IN THE OSMOTIC PRESSURE OF THE SAP OF THE DEVELOPING LEAVES OF SYRINGA VULGARIS. Roy. Dublin Soc. Sci. Proc. (n. s.) 13: 219-222.
- (4) EATON, F. M.
1927. THE WATER REQUIREMENT AND CELL-SAP CONCENTRATION OF AUSTRALIAN SALTBUSH AND WHEAT AS RELATED TO THE SALINITY OF THE SOIL. Amer. Jour. Bot. 14: 212-226, illus.

- (5) EATON, F. M., and BELDEN, G. O.
1929. LEAF TEMPERATURES OF COTTON AND THEIR RELATION TO TRANSPIRATION, VARIETAL DIFFERENCES, AND YIELDS. U. S. Dept. Agr. Tech. Bul. 91, 40 p., illus.
- (6) FERNALD, E. I.
1925. THE INHIBITION OF BUD-DEVELOPMENT AS CORRELATED WITH THE OSMOTIC CONCENTRATION OF SAP. Amer. Jour. Bot. 12: 287-305.
- (7) GORTNER, R. A., and HARRIS, J. A.
1914. NOTES ON THE TECHNIQUE OF THE DETERMINATION OF THE DEPRESSION OF THE FREEZING POINT OF VEGETABLE SAPS. Plant World 17: 49-53.
- (8) GIDDINGS, L. A.
1914. TRANSPIRATION OF SILPHIUM LACINIATUM, L. Plant World 17: 309-323, illus.
- (9) HARRIS, J. A., HOFFMAN, W. F., and LAWRENCE, J. V.
1925. DIFFERENTIAL ABSORPTION OF ANIONS BY VARIETIES OF COTTON. Soc. Expt. Biol. and Med. Proc. 22: 350-352.
- (10) HURD-KARRER, A. M.
1926. A CONCENTRATION GRADIENT IN CORN STALKS. Jour. Gen. Physiol. 9: 341-343.
- (11) KOKETSU, R.
1926. STUDIES ON THE FOLIAR TRANSPIRING POWER AND ITS DAILY FLUCTUATION AS RELATED TO THE DEVELOPMENT OF LEAVES IN COLEUS BLUMEI. Bot. Mag. [Tokyo] 40: 122-131. [In Japanese, English summary.]
- (12) LUDWIG, C. A.
1927. SOME EFFECTS OF LATE DEFOLIATION ON COTTON. S. C. Agr. Expt. Sta. Bul. 238, 23 p., illus.
- (13) MASON, T. G.
1919. ON SOME FACTORS AFFECTING THE CONCENTRATION OF ELECTROLYTES IN THE LEAF-SAP OF SYRINGA VULGARIS. Roy. Dublin Soc. Sci. Proc. (n. s.) 15: 651-666.
- (14) OVERTON, J. B.
1911. STUDIES OF THE RELATION OF THE LIVING CELLS TO TRANSPIRATION AND SAP-FLOW IN CYPERUS. I-II. Bot. Gaz. 51: 28-63, 102-120, illus.
- (15) PALLADIN, V. I.
[1923]. PLANT PHYSIOLOGY. Amer. Ed. 2, edited by B. E. Livingston, 360 p., illus. Philadelphia.
- (16) PRINGSHEIM, E.
1906. WASSERBEWEGUNG UND TURGORREGULATION IN WELKENDEN PFLANZEN. Jahrb. Wiss. Bot. 43: [89]-144.
- (17) REED, H. S.
1921. GROWTH AND SAP CONCENTRATION. Jour. Agr. Research 21: 81-98, illus.

DISEASES OF ROSE CAUSED BY SPECIES OF CONIOTHYRIUM IN THE UNITED STATES¹

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INTRODUCTION

In the examination and study of many types of cankers on diseased rose stems the attention of the writer has been attracted to the great prevalence of species of *Coniothyrium* as the cause of such cankers. Because of the many points of similarity among the three diseases popularly known as stem canker, graft canker, and brand canker, attributed respectively to *C. fuckelii* Sacc., *C. rosarum* Cke. and Hark., and *C. wernsdorffiae* Laub., the question frequently arises as to the identity of the particular organism causing a certain type of canker. The present study was undertaken to determine by a comparison of the symptoms of disease and of the morphological and physiological characters of the causal fungi what distinguishing features might be of value in diagnosing the diseases. The immediate occasion for the study was the recent discovery, in the United States, of brand canker, caused by *C. wernsdorffiae*, hitherto reported in Europe and Canada.

SURVEY OF LITERATURE

Brand canker was originally described in 1905 by Laubert (10)² as a disease attacking branches of Rosa in many localities in Germany. From a morphological study of the organism causing the canker, he concluded that the fungus was a species of *Coniothyrium*. The species, however, did not correspond with *C. fuckelii*, which had been reported by Saccardo (19) as occurring on dead or wilted branches of Rosa and other hosts. Therefore Laubert established the fungus as a new species, *C. wernsdorffiae*. Kock (9) in the same year reported a disease on tea roses in Austria which he ascribed to *C. fuckelii*. He believed that this was the same disease as that found by Laubert and that the slight difference between the spore measurements given for Saccardo's fungus and those of his own Austrian specimens was an insufficient basis for establishing a new species. He would make the fungus causing the disease merely a variety of *C. fuckelii*. His spore measurements and description of the disease symptoms corresponded rather closely with those given for *C. wernsdorffiae* by Laubert (10), and it would seem that both writers were describing the same disease.

In a later publication Laubert (11) compared the fungus causing the brand canker with *Coniothyrium fuckelii*. He considered the latter fungus a harmless saprophyte with pycnidia and spores much smaller than those of *C. wernsdorffiae*.

Güssow (4, p. 226) described the symptoms and causal organism of a similar disease found upon hybrid tea roses and Wichuraianas in

¹ Received for publication Dec. 30, 1929; issued May, 1930. These studies were conducted in cooperation with the Department of Botany, Brown University, Providence, R. I.

² Reference is made by number (italic) to "Literature cited," p. 826.

Ireland. He considered the causal organism to be *Coniothyrium fuckelii*, since his spore measurements "practically agreed with the size of the spores of Saccardo's fungus." Moreover, he believed that the disease on the Irish specimens was identical with that observed by Laubert and agreed with Kock that Laubert was not justified in establishing a new species. From Güssow's description and the accompanying illustrations it would seem very probable that his specimens were infected by *C. fuckelii*, as he stated, and that he was not dealing with the disease described by Laubert.

In Rostrup's Danish Fungi (13, p. 436) appeared the following note concerning *Coniothyrium wernsdorffiae*: "A true parasite, attacking the bark of the branches of cultivated *Rosa* spp. for the first time found 11/6/03 [June 11, 1903], quite common."

O'Gara (16) found a species of *Coniothyrium* causing cankers on rose in the United States which proved by cultural methods and cross inoculations to be identical with a species occurring on cankers on apple twigs. The fungus appeared to correspond in every respect with the description of *C. fuckelii* given by Saccardo. O'Gara stated that the organism fruited readily in cultures, "producing typical pycnidia and spores varying somewhat in size, depending upon the medium, but all within the limits of the species."

From these investigations it seemed apparent that two different species of *Coniothyrium* caused diseases of rose. Since the time of O'Gara's investigations, however, several writers have stated that *C. wernsdorffiae* was probably identical with *C. fuckelii*.

Saccardo (19) designated *Coniothyrium fuckelii* as the "spermagonial" form of *Leptosphaeria coniothyrium* (Fckl.) Sacc. The same statement was made by Massee (15). Experimental inoculations and cultures to determine the exact relation of the perithecial and pycnidial forms were successfully made by Stewart (20) on various species of *Rubus*. Martin (14), in a discussion of the polymorphism of *L. coniothyrium* on rose, reported *Coniothyrium* as one of the phases of the fungus. So far as known at the present time no perfect stage for *C. wernsdorffiae* has been reported.

In 1925 the first reports of the occurrence of brand canker in North America were published by Howitt (6) and by Martin,³ following the identification of *Coniothyrium wernsdorffiae* as the cause of cankers on material collected in Guelph, Ontario. Later the occurrence of the disease in the United States was reported by Jenkins and Martin (8)⁴ and was tentatively described by Jenkins (?), Westcott,⁵ and the writer (23). Its occurrence in the Netherlands (18) was also reported.

The third disease of rose attributed to a species of *Coniothyrium* was described by Vogel (22) as graft disease. From his study of the causal fungus and from the results of his inoculation experiments he concluded that the disease was caused by *C. rosarum*. This fungus was originally reported by Cooke and Harkness (2) as occurring on stems of *Rosa* in California. No report of the perfect stage of this fungus has been made.

³ MARTIN, G. H. DISEASES OF FOREST AND SHADE TREES, ORNAMENTAL AND MISCELLANEOUS SHRUBS IN THE UNITED STATES IN 1924. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rprtr. Sup. 42: 360. 1925. [Mimeographed.]

⁴ JENKINS, A. E., and MARTIN, G. H. BRAND CANKER OF THE ROSE APPEARS IN THE UNITED STATES. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rprtr. 10: 24-25. 1926. [Mimeographed.]

⁵ WESTCOTT, C. AN EPIPHYTIC OF BRAND CANKER OF ROSES CAUSED BY CONIOTHYRIUM WERNSDORFFIAE LAUBERT. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rprtr. 10: 38-40. 1926. [Mimeographed.]

DISTRIBUTION OF THE DISEASES

In the summer of 1926 a serious epiphytotic of a disease similar to that originally described in Germany by Laubert as brand canker caused by *Coniothyrium wernsdorffiae* was discovered by the writer on climbing roses in the floriculture gardens at Cornell University, Ithaca, N. Y. (Fig. 1.) No report of the occurrence of this disease in the United States had been published at that time. Later the disease was reported by Jenkins and Martin (8) as occurring also in Minnesota and Pennsylvania. So far as known at the present time, this disease occurs principally on out-of-door roses. Jenkins and Martin (8) reported it on rose plants grown in the horticulture greenhouses of the Minnesota Agricultural College. With this exception all reports and collections have been from garden plants.

The disease commonly known as stem canker (fig. 2) and caused by *Coniothyrium fuckelii* is reported as widespread throughout the United States. It is found at some time in nearly every rose garden and in many greenhouses. It is more prevalent, however, on hybrid tea roses than on other types of roses.

The so-called graft disease attributed by Vogel to *Coniothyrium rosarum* was considered to be limited to rose grafts in forcing frames and greenhouses and to plants in the rose garden which had contracted the disease while in the greenhouse but which proved to be partially resistant. During 1927 a similar disease (fig. 3) was particularly prevalent in greenhouses throughout the middle and eastern parts of the United States.

The accompanying map (fig. 4) shows the distribution of these three diseases in the United States as known at the present time. This map was prepared from data published by Martin and Jenkins⁶ and from the results of the writer's studies. Undoubtedly the diseases are more widely distributed than this map signifies, but possibly they are not sufficiently serious to attract particular attention in those States from which a report of their occurrence has not been received.



FIGURE 1.—Brand canker, showing fruiting bodies of the causal fungus

⁶ MARTIN, G. H., and JENKINS, A. E. PRELIMINARY LIST OF FUNGI AND DISEASES OF ROSES IN THE UNITED STATES. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rpt. 63:357, 1928. [Mimeographed.]

MATERIAL USED IN THE PRESENT STUDY

The material used in the present study was collected from various localities in the United States and from plants grown under widely differing conditions of environment. The specimens of brand canker



FIGURE 2.—Stem canker which started from an infection of the wound at the lower end of the prickles

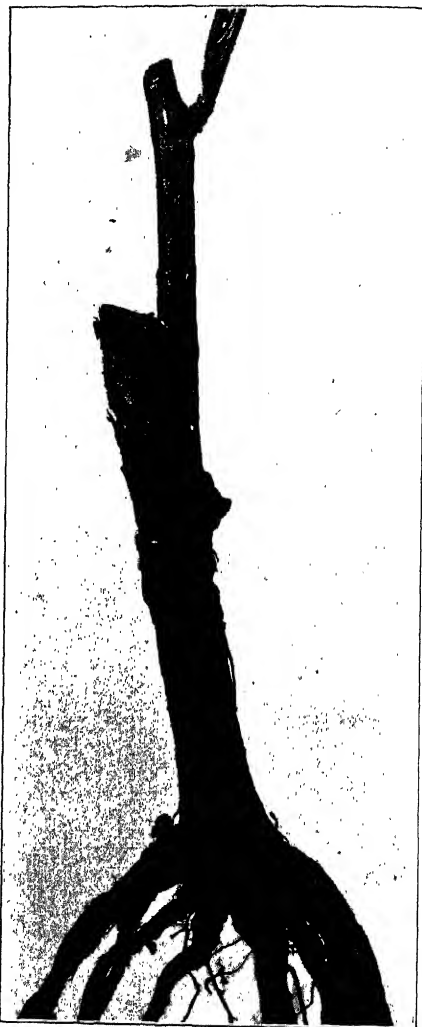


FIGURE 3.—A young grafted rose plant bearing graft canker along the side of the scion nearest the stock

were collected by the writer at Ithaca, N. Y., and were a portion of the material reported by Jenkins and Martin (8), who attributed the disease to *Coniothyrium wernsdorffiae*. The term "stem canker" is

applied throughout this study to the disease which is commonly considered to be caused by *C. fuckelii*, the imperfect form of *Leptosphaeria coniothyrium* Sacc. A few specimens of *L. coniothyrium* were studied by the writer and were compared with exsiccati specimens among the "Fungi Saxonici" collected by K. W. Krieger. In the study of this material, cultures from the ascospores produced a species of *Coniothyrium* which corresponded with that isolated from the stem cankers. Therefore the fungus causing such cankers has been called *C. fuckelii*. The name "graft canker" has been applied only to the disease characterized by the formation of cankers at the union of stock and scion. The fungus isolated from such cankers was compared with that occurring on specimens from the herbarium of the Iowa State College of Agriculture and Mechanic Arts,⁷ which were collected by I. H. Vogel, who attributed the disease to *C. rosarum*.

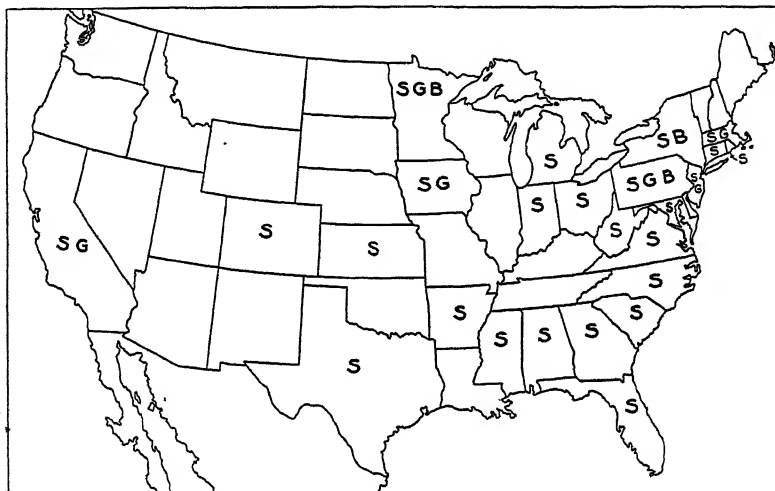


FIGURE 4.—Map showing distribution of stem canker, graft canker, and brand canker diseases in the United States. S=stem canker; G=graft canker; B=brand canker

BRAND CANKER

SYMPTOMS

The symptoms of the disease known as brand canker have been described in detail by various investigators (9, 10, 11, 23). Therefore it is necessary in this article only to call attention to those characters which are distinguishing features.

The cankers can be readily detected, even by a casual observer. This is due to the fact that the light or warm buff⁸ of the central portion and the taupe-brown or dull purplish black margin stand out in sharp contrast with the green of the adjacent healthy stem.

Small longitudinal slits in the bark of the diseased area, caused by the protruding of the pycnidial ostioles, are characteristic of these cankers. Only in rare cases were the spores of the fungus extruded

⁷ The writer acknowledges the courtesy of Dr. I. E. Melhus and Dr. J. C. Gilman, of the Iowa State College of Agriculture and Mechanic Arts, for the loan of this material.

⁸ The color terms mentioned in the text are according to the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

upon the bark in sooty masses. In Laubert's description of the disease (10), however, he stated that the spores are frequently collected on the surface of the bark "in Form sehr kleiner russschwarzer Fleckchen." Since the majority of the cankers of this disease which were examined by the writer were of comparatively recent infection, it may be that the accumulation of the spores occurs only on older cankers. Laubert also mentions the occurrence of wound callus at the margin of 2-year-old cankers (10). No such callus formation has been found in connection with the cankers examined by the writer. This again may be due to the fact that young cankers only have been observed in this country.

MORPHOLOGY OF THE CAUSAL FUNGUS

MYCELIUM

In the host tissue the mycelium of the causal fungus showed no distinguishing features. In culture, however, although the mycelium varied with the kind of agar used, certain distinguishing features of color and amount of growth were apparent. The aerial mycelium of the fungus grew exceedingly slowly on all the media used but eventually became luxuriant. On corn-meal and potato agars it was light gray; on malt, dextrose, and synthetic ⁹ agars, a greenish gray turning to brownish gray. On sterilized sliced potato, the actively growing mycelium was a very light gray, becoming darker with age until, in an old culture, it was almost black.

FORMATION OF PYCNIDIA AND SPORES

The formation of a pycnidium is first indicated by close interweaving and anastomosing of numerous rapidly growing hyphae. This usually occurs in some intercellular space at a point within the zone of the three outermost cell layers below the epidermis of the diseased area. As this tangled mass or primordium grows larger, the epidermis together with the two or three underlying cell layers becomes widely separated from the inner layers of the bark tissue. In the center of the primordium a cavity is formed by the absorption of some of the hyphal cells. By this lysigenous activity the cavity is gradually increased in area until it attains its characteristic size. In a mature pycnidium the cells lining the cavity are hyaline, thin walled, and finely granular. These cells are sporogenous in function. There is no pigmentation in the cell walls of this tissue. The next two or three layers are similar to these in appearance. These few layers of sporogenous cells are closely packed and more or less regular in shape. This arrangement, together with the granular contents of the cells, gives the appearance, in sections, of a subhyaline ring of tissue surrounding the cavity. This ring is especially characteristic of the pycnidia formed by the brand-canker fungus. (Fig. 5.) Adjacent to these layers are several rows of larger cells, devoid of granular cell contents and containing a brown pigmentation in the cell walls. These layers of cells form the enveloping wall of the pycnidium.

The pycnidial cavity may vary considerably in shape, and the pycnidia are usually so closely grouped that they appear stromatic.

⁹ The synthetic agar was made according to the formula given by Leonian (18) as follows: Dihydrogen potassium phosphate, 1.25 gm.; magnesium sulphate, 0.625 gm.; peptone, 0.625 gm.; maltose, 6.25 gm.; malt extract, 6.25 gm.; distilled water, 1,000 c. c.; agar, 1.5 to 2 per cent.

(Fig. 6.) The fungus is characterized by complex pluriloculate pycnidia rising from several confluent primordia. Mature pycnidia are frequently found in which the intervening walls between the aggregate pycnidia have broken down, leaving one large irregular cavity.

In Laubert's description of *Coniothyrium wernsdorffiae* (10, p. 460) he described the pycnidia as "mit ektostromaartiger Papille," penetrated by a broad ostiolate canal. From a study of sections from a number of pycnidia on the host tissue it was found that, when the formation of the pycnidial cavity is completed, there develops at the top of the pycnidium, just below the epidermis, a small cone-shaped mass of thin-walled cells forming a "buffer tissue" (3). (Fig. 5.) The walls of these cells are at first hyaline, but as the tissue grows and finally ruptures the epidermis, the walls become subhyaline, then brown, and finally almost black. The cells along the central line of

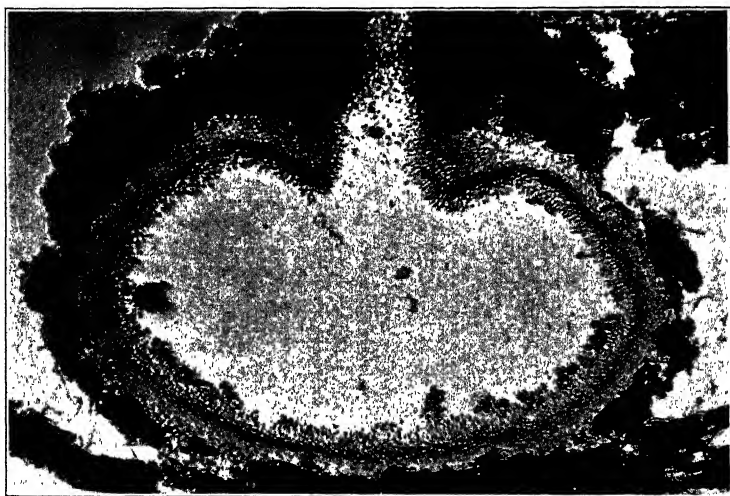


FIGURE 5.—Pycnidium of the brand-canker fungus showing the ostiole developing through the buffer tissue and the apparent ring of tissue formed by the layers of sporogenous cells. $\times 160$

this tissue dissolve or disintegrate, and a canal ending in a papillate ostiole results. From the cells lining this canal are produced short filamentous cells resembling the periphyses commonly found in papillate or beaked ostioles of perithecia. These periphyses may disappear as soon as the pycnidium becomes mature, but the buffer tissue often remains for some time. It is this buffer tissue which caused Laubert to describe the pipilla as "ektostromaartige."

In culture the pycnidia show more distinctly than in nature the beaklike papilla through which the ostiolar canal develops. The buffer tissue, however, was present only preceding the formation of the papilla and ostiole. It was frequently found that a secondary cavity was formed in the beaklike papilla. (Fig. 7.) The two cavities are at first separated from each other by a wall several cell layers in thickness. Eventually, however, the separating wall is broken down and an ostiole is formed through the outer wall of the upper cavity.

Laubert (10) further stated that the spores were abstricted from the innermost layer of cells in the pycnidial cavity and that sporophores were lacking. The manner in which the spores are produced in certain species of *Coniothyrium* has been considered by Von Höhnelt



FIGURE 6.—Aggregate pycnidia of the brand-canker fungus. $\times 160$

(5) as the basis for separating these species of the genus into a new genus, *Sclerothyrium*. The distinguishing characters of this new genus are the absence of conidiophores and the endogenous formation of the spores.

In the present study of the brand-canker fungus it has been found that the spores are produced by budding from the innermost layer of cells lining the pycnidial cavity. (Fig. 8, A.) These cells appeared to be coated with a gelatinous substance, probably resulting from the lysigenous activity in the process of cavity formation. The first indication of spore formation is the protrusion of a very slender

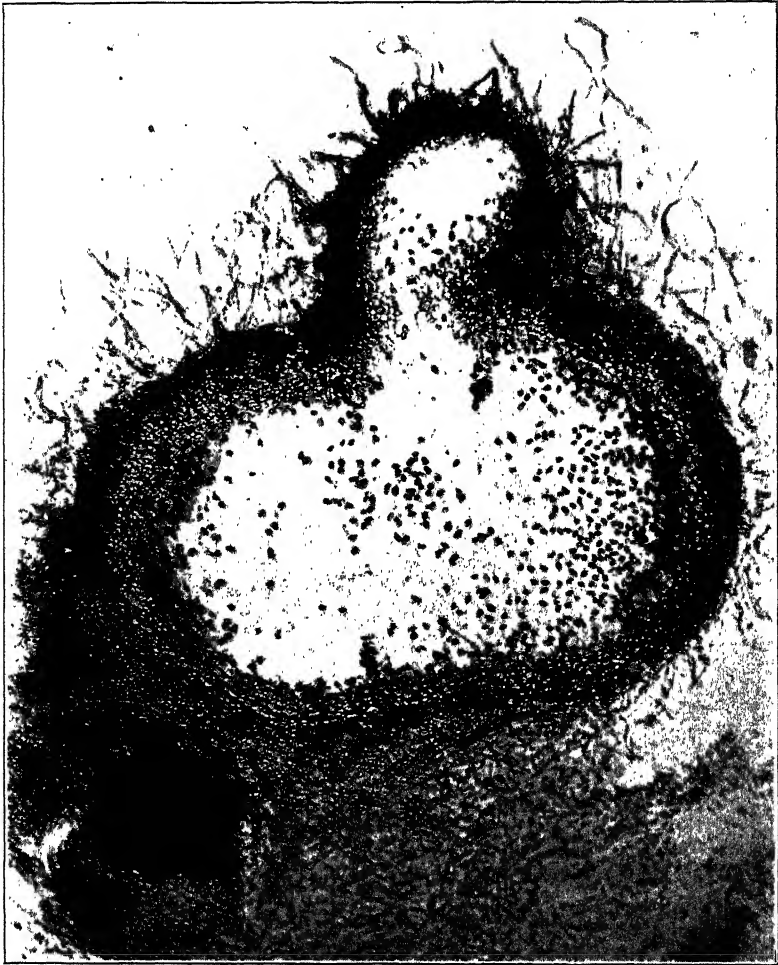


FIGURE 7.—A pycnidium of the brand-canker fungus produced in culture. A secondary cavity has formed in the beaklike papilla. $\times 160$

filament of cytoplasm from a cell of the sporogenous layer into the gelatinous coating. The tip of this filament gradually swells and finally emerges from the coating. It then has the ovoid or spherical form of a mature spore and has a thin gelatinous film surrounding it. This ovoid tip remains connected with its parent cell by a thin strand of cytoplasm until the spore reaches maturity. A wall is then formed between the spore and its parent cell and the cytoplasmic strand

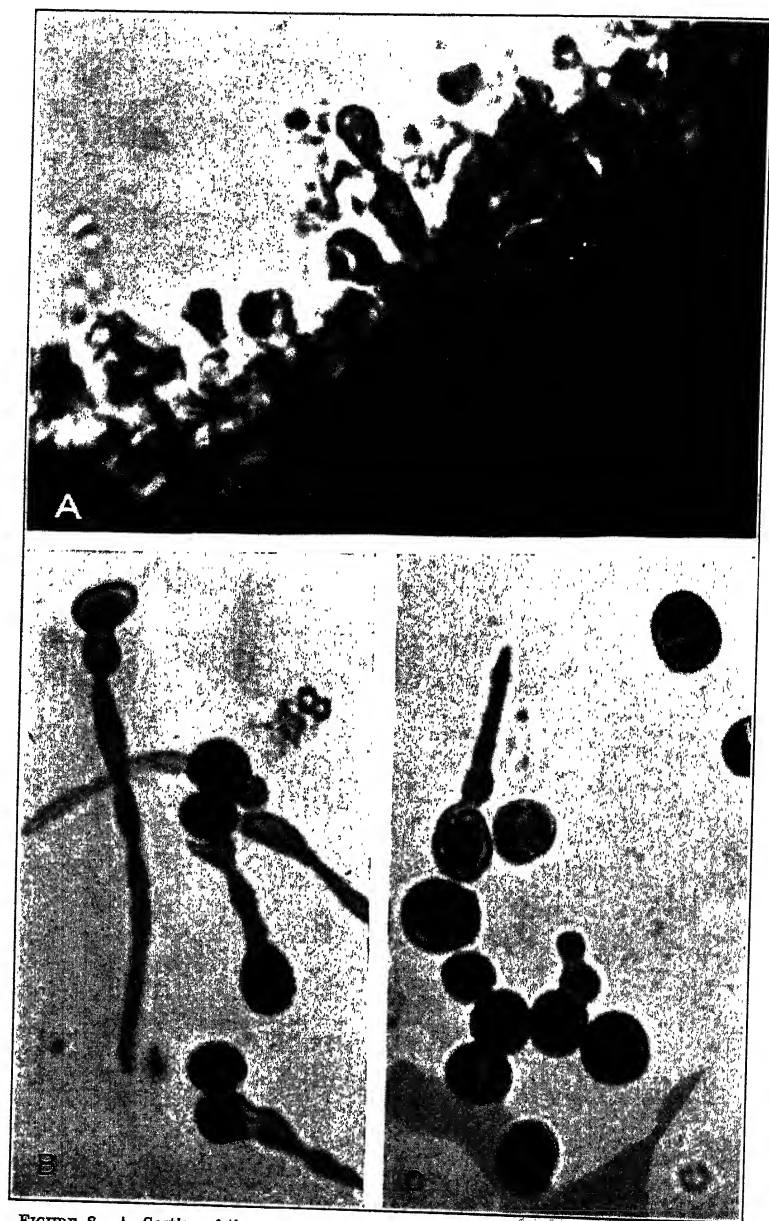


FIGURE 8.—A, Section of the sporogenous layer of cells in a pycnidium of the brand-canker fungus, showing near the center a developing spore. $\times 2,500$. B, Germinating spores of the brand-canker fungus. $\times 1,300$. C, Spores of the brand-canker fungus which became septate before germination. $\times 1,300$

disappears. This type of spore formation has been described for certain species of both *Phoma* (1, 24) and *Coniothyrium* (17), which were placed respectively in the genera *Sclerophoma* and *Sclerothyrium* by Von Höhnelt (5). It is apparent that the spores are not formed endogenously, nor are they produced on typical conidiophores. It seems advisable, however, to designate the species as of the genus *Coniothyrium* until a revision of Von Höhnelt's *Endogenosporae* has been made.

The ovoid spore remains hyaline until it reaches its mature size, at which time pigmentation begins to appear. The pigmentation is confined to the exospore of the spore wall. The spore contents remain colorless.

The spores are abstricted singly from the parent cells. Because of the gelatinous film surrounding each spore, they frequently adhere end to end and give the appearance of chains of spores produced from one cell.

SIZE AND SHAPE OF SPORES

The question of spore size has been considered of importance in determining whether the three rose diseases are caused by the same or different species of *Coniothyrium*. Laubert's measurements for *C. vernsdorffiae* (10) are given as 4.5μ to 6μ by 5μ to 8μ . Kock (9) found that the spores of his specimens gave an average length of 5.6μ and width of 4μ . The length of the spores was very variable, some as long as 5.7μ , others 6.6μ , and still others 4.7μ .

In the present study spores of the brand-canker fungus on the host were obtained by crushing on a slide several mature pycnidia. The spores were mixed thoroughly, and a few loopfuls were mounted on a slide in a solution of potassium acetate, 10 gm.; pure glycerine, 200 c. c.; 95 per cent alcohol, 300 c. c.; distilled water, 500 c. c. The spores were allowed to stand in a drop of the solution on a slide for 24 hours, and 50 spores from each of two slides were then measured with a filar micrometer. The measurements of the two sets were then combined, and the means and standard range for the 100 spores were calculated. One hundred spores from several pycnidia produced in polysporic cultures on potato agar were similarly measured. Extruded spores only were used for this series of measurements. The origin of the material used and the results obtained are shown in Table 1. The mean ratio of length to width is given in Table 2.

TABLE 1.—*Biometric data on spores of fungi from brand canker, stem canker, and graft canker*

Fungus	Source of spores	Length			Width		
		Mean (μ)	Standard deviation	Standard range (μ)	Mean (μ)	Standard deviation	Standard range (μ)
Brand canker....	Host; garden; New York....	5.29	0.533	4.76-5.82	3.83	0.36	3.47-4.19
Do.....	Culture.....	5.61	.667	4.94-6.28	4.18	.385	3.79-4.57
Stem canker....	Host; garden; District of Columbia.	3.09	.358	2.73-3.45	2.15	.26	1.89-2.41
Do.....	Host; garden; Rhode Island.	2.98	.427	2.55-3.41	2.01	.276	1.73-2.29
Do.....	Host; grafted plants; greenhouse; Georgia.	3.27	.405	2.87-3.68	2.18	.281	1.89-2.46
Do.....	Host; cuttings; greenhouse; Maryland.	3.37	.436	2.93-3.81	2.16	.292	1.87-2.45
Do.....	Culture.....	2.75	.368	2.38-3.12	2.02	.41	1.61-2.43
Graft canker....	Host; greenhouse; Pennsylvania.	3.04	.426	2.61-3.47	2.17	.296	1.87-2.47
Do.....	Host; greenhouse; Iowa....	3.18	.389	2.79-3.57	2.17	.273	1.89-2.44
Do.....	Culture.....	3.2	.376	2.82-3.58	2.13	.234	1.89-2.36

TABLE 2.—Ratio of length to width for spores of fungi from brand canker, stem canker, and graft canker

Fungus	Source of spores	Mean ratio
Brand canker.....	Host.....	1.35
Do.....	Culture.....	1.32
Stem canker.....	Host.....	1.47
Do.....	Culture.....	1.35
Graft canker.....	Host.....	1.39
Do.....	Culture.....	1.49

The actual range of the spores measured was 4.0μ to 7.2μ by 3.0μ to 5.4μ . There is a slight difference between these measurements and those given by Laubert (10) for *Coniothyrium wernsdorffiae*. This is probably due to the fact that spores larger than 7.2μ are exceedingly rare. Many pycnidia from cultures were examined, the pycnidia being crushed on a slide in an attempt to force out all the spores. In one case where two large pycnidia were crushed on a slide, only 12 spores on the entire slide could be found whose length exceeded 7.2μ ; these ranged from 7.4μ to 9.4μ . Another slide with two crushed pycnidia showed a larger number of spores exceeding 7.2μ in length. The largest spore measured was 10.6μ by 10.4μ . The width also varied in these larger spores. Their actual measurements may be given as 7.4μ to 10.6μ by 5.2μ to 10.4μ . Among the spores on the slide, however, were many that had begun to germinate. It is therefore possible that the large size of these spores is merely a phenomenon of germination and that these measurements do not indicate the normal size of a mature spore. Some of these large spores were uniseptate. As will be mentioned later, this septation sometimes occurs preceding germination.

PHYSIOLOGY OF THE CAUSAL FUNGUS

DESCRIPTION OF CULTURES ON VARIOUS MEDIA

MALT AGAR.—The fungus produced a moderately abundant, fluffy, aerial mycelium which in mass was greenish gray. Pycnidia were rare and were first noticeable about four weeks after inoculation. Another three or four weeks of growth was necessary to bring them to maturity.

CORN-MEAL AGAR.—A slight amount of grayish mycelium was produced. Only a few pycnidia were formed. The rate of growth was relatively the same as on malt agar.

POTATO AGAR.—The mycelium was moderately abundant, white to mouse gray, and mature pycnidia were present about six weeks after inoculation. This medium proved very satisfactory for the production of pycnidia and spores.

STERILIZED POTATO SLICES.—A dense, fluffy, mouse gray to dark gray aerial mycelium was formed, producing abundant mature pycnidia in six to eight weeks after inoculation. The pycnidia were large with a pronounced tendency to aggregate or become complex. The spores were exuded in the form of spore horns.

DEXTROSE AGAR.—The dextrose agar was made with 2 per cent dextrose and 2 per cent agar. A moderate amount of greenish gray aerial mycelium was produced, but no fruiting bodies were developed to maturity. A few appeared to form in the mycelium along the edge of the agar slant, but they failed to develop spores.

SYNTHETIC AGAR.—The synthetic medium developed by Leonian (12) for the production of pycnidia in cultures of Sphaeropsidales was used. The medium was more satisfactory than the others with the exception of the sliced potato. A fluffy, greenish gray mycelium was formed, and pycnidia appeared after about eight weeks.

ROSE-STEM AGAR.—A medium made from a decoction of rose stems with agar was tried, but the results did not prove of value. No mature pycnidia were produced.

STERILIZED ROSE STEMS.—Stems of climbing and hybrid tea roses were cut into convenient lengths, placed in flasks with 10 to 20 c. c. of distilled water, and sterilized. When cool the stems were inoculated with mycelium, with spores, or with entire pycnidia. Growth was very satisfactory, and pycnidia were produced abundantly.

Spore Germination

Germination tests were made on several different media. In hanging-drop cultures with tap water the spores showed evidence of germination in 24 to 36 hours. (Fig. 8, B.) In distilled water the number of germinating spores was very much smaller and the rate of germination was very much slower. Tests were also made by sowing spores on a thin film of agar on a microscope slide. The agar mounts were kept moist in a Petri dish and were examined at 24-hour intervals. The agars used were malt, potato, dextrose, and Leonian's synthetic. The spores germinated on all these agars with about the same readiness as in tap water.

Germination was preceded by a swelling of the spores. Very frequently this increase in size was followed by septation. (Fig. 8, C.) The first septum usually originated as a slight separation of the cytoplasm near the center of the spore. The separation gradually extended outward to the spore wall, and a definite septum then became apparent. In some cases the spores became constricted at the septum. A second septum sometimes appeared, either parallel with or at right angles to the first septum. In these septate spores germination may take place from each of the cells. The tendency of spores of *Coniothyrium* species to become septate has been previously reported by Archer (1), but no extensive investigations have been conducted to determine the possible relation of septation to changes in environment. It is apparent, however, that spores of certain species of *Coniothyrium* may show the same tendency toward septation as do those of species of *Sphaeropsis*.

PATHOGENICITY OF THE ORGANISM

INOCULATION EXPERIMENTS

Since facilities for growing roses for inoculation under greenhouse conditions were not available to the writer, it was necessary to devise other means for growing rose cuttings. Several pots of sterilized sand were prepared, and in these pots were placed cut stems of a climbing rose. The leaves were removed and the cuttings were allowed to grow about one week before they were inoculated. One series of inoculations was made by placing the inoculum, composed of a portion of a mature pycnidium with exuding spores, directly in the axil of a bud without injuring the bud or bark in any way. In a second series the inoculum was introduced into the stem tissue through a wound in the bark made by a sterile scalpel. The inoculum used was obtained from polysporic cultures grown on sterilized potato slices. In each series of experiments one pot with a cutting was set aside as a control. In all cases the cuttings were kept in a moist atmosphere under bell jars.

All the cuttings produced new healthy leaves from the two uppermost buds. With the exception of those buds in the axils of which the inoculum was placed, the buds remained green and healthy, although they developed no leaves. The inoculated buds soon turned brown and in about six weeks were found to be covered with pycnidia. The spread of the fungus in the bark tissue, however, was very slow. The infected bark at first became a reddish brown, then a dark brown. As

the pycnidia appeared and the tissue of the affected areas became dried and dead, the color of the central portion changed to a light brown with a margin of reddish brown. Before the cankers had reached any considerable size, however, the cuttings died. The fungus was re-isolated from the infected areas. On the canes that were inoculated through wounds made with a sterile scalpel, the infected areas increased in size more rapidly and pycnidia were produced more abundantly.

At the same time a series of similar inoculations was made on red-raspberry canes. In all cases small cankers appeared on the bark of the canes before the death of the cuttings. As the bark became dried following infection, it peeled off in shreds. This characteristic was noted by Laubert (10) in cankers on rose canes caused by *Coniothyrium wernsdorffiae*. The small prickles on the raspberry canes became infected and in some cases a pycnidium was formed within the prickle, the fungous hyphae thus taking the place of the disintegrated host tissue. Cankers were formed from infected buds as well as from inoculated wounds.

No reference in literature could be found to the occurrence of *Coniothyrium wernsdorffiae* on parts of rose plants other than stems or branches. Therefore, tests were made with hybrid tea and climbing rose leaves, as well as with the hips of *Rosa rugosa*, to determine whether the fungus would infect them and possibly overwinter in this way. In no case, however, did the fungus infect the leaves or hips in a moist chamber.

PENETRATION OF THE HOST

In Laubert's (10) discussion of the symptoms of brand canker caused by *Coniothyrium wernsdorffiae*, he stated that the majority of cankers were found around the dormant buds. This is an especially noticeable symptom of the disease in the United States. Of the many cankers examined, the majority appeared to have had their origin in the vicinity of a dormant bud. A few, however, originated without a wound or a bud as the locus of infection.

The results of the inoculation experiments in the present study indicate that the fungus is capable of infecting dormant buds. Growth was more rapid, however, when the germ tubes were able to gain entrance through wounds.

STEM CANKER AND GRAFT CANKER

In Vogel's discussion of the rose-graft disease (22) his conclusions as to the identity of the causal fungus were based upon his inoculation experiments, the size of the spores and pycnidia, and the comparison of cultures of the organism found on rose with those of *Coniothyrium fuckelii* isolated from black-raspberry canes. He stated that "a characteristic symptom of this disease is the occurrence of lesions on the scion at the union, and just above the union." In the present study, therefore, only those specimens which showed definite cankers at the union of stock and scion were considered as affected with the graft disease. The fungus causing these cankers was closely compared with that occurring on the so-called stem cankers. Therefore the symptoms, morphology, and physiology of these two fungi will be considered together.

SYMPTOMS

In the case of well-developed graft cankers, the color is darker than that of stem cankers. It varies from Dresden or cinnamon brown¹⁰ at the center to hazel or auburn at the margin. The main portion of the stem cankers, however, varies in color from wood brown or cinnamon buff to snuff brown or Saccardo's umber. The margin is frequently army brown.

In both cases the presence of the fungus can be detected by means of a hand lens because of the sooty covering of spores over the slightly erumpent pustules. A splitting of the bark along the margin of the canker and the succeeding callus formation are characteristic of both of these diseases.

MORPHOLOGY OF THE CAUSAL FUNGI

MYCELIUM

The mycelia in the tissue of the two types of cankers appeared to be similar. No distinguishing features could be observed. This also was true of the mycelia developed in cultures. On corn-meal agar the mycelia were comparatively scanty and were white without any conspicuous tinge of gray. The gray was more pronounced, however, on the dextrose and synthetic agars, on which also the aerial mycelia were luxuriant. The mycelia on malt agar were not so luxuriant and were a slightly darker gray. On potato agar and sterilized potato slices the mycelia were very luxuriant and the color varied from white to light gray.

FORMATION OF PYCNIDIA AND SPORES

The pycnidia and spores of both the graft-canker and stem-canker fungi were formed similarly to those of the brand-canker fungus. In the case of the graft-canker fungus, however, the majority of the pycnidia on the host were simple globose unilocular structures. (Fig. 9.) The stem-canker fungus appeared to form complex pluriloculate pycnidia arising from several confluent primordia. (Fig. 10.) In this respect it resembled the brand-canker fungus.

In Saccardo's description of *Coniothyrium fuckelii* (19) he stated that the papillate ostiole is scarcely prominent. Thomé (21) described the pycnidia of the same fungus as having an inconspicuous, scarcely prominent papillate ostiole. The original description of *C. rosarum* by Cooke and Harkness (2) did not mention the type of ostiole or the method of spore discharge. In the diagram given by Vogel (22) the spores of his *C. rosarum* appear to be exuding through a pore in the upper wall of the pycnidium. This method of spore discharge would correspond with that observed by Archer (1, p. 51) in his study of *C. concentricum*. He stated that "no definite ostiole is formed but it seems that usually there is merely a rupture or dissolution in the upper wall, at a point just below a stoma, which allows for the discharge of the spores."

A papillate ostiole was found in the pycnidia of both the fungi studied. The manner of its formation and its general character were similar. A buffer tissue was developed, as in the pycnidia of the brand-canker fungus, with an ostiolate canal and periphyses. The buffer tissue was not so conspicuous, however, and both buffer tissue

¹⁰ RIDGWAY, R. Op. cit.

and periphyses became disorganized when the pycnidia reached maturity and thus eventually disappeared. The ostiolate canal was very short, and, as Thomé (21) stated, the papilla scarcely breaks through the epidermis.

In culture the pycnidia were developed in much the same manner as on the host. No buffer tissue, however, was found in connection with either fungus. The first pycnidia formed were usually simple uniloculate structures. Later both fungi developed complex pluriloculate pycnidia arising from confluent primordia. Vogel (22) stated that in culture the pycnidia of his *Coniothyrium rosarum* were larger than those of his *C. fuckelii* isolated from black raspberry. It is possible that he was comparing the complex pycnidia of one fungus with the simple pycnidia of the other.

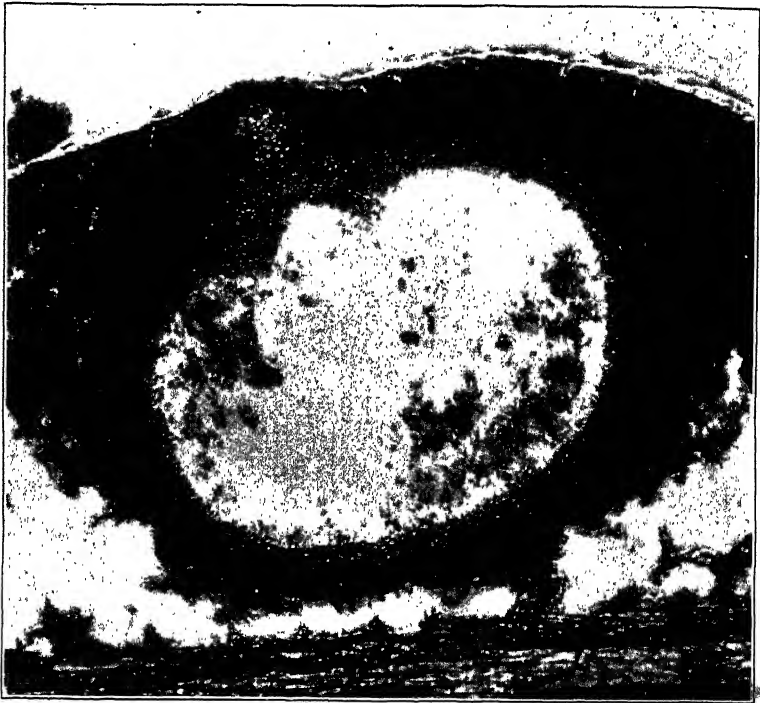


FIGURE 9.—A simple unilocular pycnidium of the graft-canker fungus. $\times 200$

Saccardo (19) described *Coniothyrium fuckelii* as "basidiis non visibilibus." Thomé (21), in his description of the same fungus, stated that the conidiophores are not perceptible. No mention of the conidiophores is made by Cooke and Harkness (2) in connection with *C. rosarum*. Vogel also (22) does not mention them, and from the diagram included in his article it would appear that he failed to find them.

The spores obtained from the two types of cankers studied by the writer were formed by budding, in exactly the same manner as those of the brand-canker fungus. No evidence of an endogenous spore origin could be found.

SIZE AND SHAPE OF SPORES

Vogel (22), in the discussion of his *Coniothyrium rosarum*, distinguished this species from *C. fuckelii* as follows:



FIGURE 10.—Aggregate pycnidia of the stem-canker fungus. $\times 160$

The spores [*C. rosarum*] are single celled, globose to ellipsoid, brown, 3μ by 3μ to 4μ in size. These measurements are the same as given by Cooke and Harkness for *Coniothyrium rosarum*. * * *

The measurements of *C. fuckelii* given by Massee are, pycnidia 180μ to 200μ in diameter; spores 3μ to 4μ by 2μ to 3μ .

Saccardo (19) reported the spore measurements of *Coniothyrium fuckelii* as 2.4μ to 5μ by 2μ to 3.5μ , and of the variety of *C. fuckelii* on Rosa as 3μ to 5μ by 3μ . The description of *C. fuckelii* given by O'Gara (16) corresponds with that by Saccardo except for the spore measurements, which he gave as 2μ to 4.5μ by 2μ to 3.5μ . The difference in the measurements given for spore length might be explained as the result of an accidental transposition of the decimal point and the figure 4.

It was impossible in the present study to formulate a plan for measuring the spores produced by the fungi from the two types of cankers which would eliminate from consideration the modifying influence of all contributory environmental factors. The fact that the so-called graft canker is produced typically on young grafts under greenhouse conditions and the stem canker on older plants in either the greenhouse or the garden creates difficulties in making a satisfactory comparison. Moreover, the smallness of the spores and the difficulty of selecting for measurement spores of equivalent maturity add to the possibility of inaccuracies in the calculation and comparison of spore size. For these reasons it is evident that the data on spore measurements here presented do not constitute a comprehensive biometric study, but show the results obtained from certain specimens grown under the conditions stated.

The spores were measured according to the procedure already described for the spores of the brand-canker fungus. In order to increase visibility, a stain consisting of 10 gm. of erythrosin was added to the mounting medium. The source of the material and the results obtained are given in Table 1, and the ratio of length to width in Table 2.

In the actual measurements the spores were found to vary considerably in both length and width. The actual range of the spores was as follows: Graft-canker fungus, 2.0μ to 4.0μ by 1.6μ to 3.6μ ; stem-canker fungus, 2.0μ to 4.4μ by 1.2μ to 3.0μ . It is apparent that these measurements, respectively, show the same range as those of Cooke and Harkness for *Coniothyrium rosarum* and of Massee for *C. fuckelii*. As in the case of the brand-canker fungus, spores larger than those of the ranges given were exceedingly rare and were usually found only in mounts bearing germinating spores.

PHYSIOLOGY OF THE CAUSAL FUNGI

DESCRIPTION OF CULTURES ON VARIOUS MEDIA

Comparative series of cultures were made of the fungi isolated from the two types of cankers. Specimens of the cankers were placed in moist chambers, and as soon as the spores exuded from the pycnidia in a gelatinous mass a few of them were transferred to the culture tubes. Transfers were always made on the same day and under conditions of environment as similar as possible. As previously stated, no distinguishable differences could be detected between the organisms from the two types of cankers in either the mycelial growth or the production of pycnidia. The characteristics of growth upon the various media were as follows:

MALT AGAR.—The young mycelium was almost white, that is, just barely tinged with gray. As development proceeded the aerial mycelium became

moderately abundant and fluffy with a more decided grayish tinge. The young advancing mycelium, however, appeared white. In about two weeks after inoculation the pycnidia began to develop. They were produced in moderate abundance and were usually single. In an old culture, however, the tendency toward aggregate pycnidia was noticeable.

CORN-MEAL AGAR.—A small amount of white mycelium was produced, which was more closely matted than on malt agar and showed no conspicuous tinge of gray. Pycnidia were developed in abundance and were at first a yellowish brown, deepening to dark brown at maturity. The black sooty mass of spores at the ostiole was particularly noticeable. The rate of growth was relatively the same as on malt agar.

POTATO AGAR.—The mycelia in all the cultures on potato agar were white or very light gray and moderately abundant. Numerous dark-brown fruiting bodies were formed. The rate of growth was slightly more rapid than on the other media.

STERILIZED POTATO SLICES.—This medium proved very satisfactory for the production of both mycelium and fruiting bodies. Dense white aerial mycelia and complex pycnidia were formed in all the cultures.

DEXTROSE AGAR.—As in the case of the brand-canker fungus, the mycelial growth was medium in amount. Comparatively few pycnidia were formed in the grayish-white mycelium.

SYNTHETIC AGAR.—Excellent cultures resulted from the use of this medium. The mycelium was abundant, fluffy, and white slightly tinged with gray. Good-sized pycnidia were produced in moderate amount, both single and aggregate structures being found.

ROSE-STEM AGAR.—The organism produced only a moderate growth; a few pycnidia being developed to maturity.

STERILIZED ROSE STEMS.—Portions of rose stems were prepared as already described in connection with the brand-canker fungus, and were inoculated with spores, mycelium, or pycnidia of the organisms from the graft cankers and the stem cankers. This method was the most successful for obtaining an abundance of mature pycnidia and spores.

SPORE GERMINATION

Germination experiments, comparable to those for the brand-canker fungus, were made in tap water, distilled water, and the various media. The spores from both cankers germinated readily after 24 hours in tap water, but not quite so readily in distilled water. Moreover, the percentage of germination was greater in tap water than in distilled water. On the agars used the rate and the percentage of germination gave results similar to those from the hanging-drop cultures in tap water. In all cases a pronounced swelling of the spore preceded germination. (Fig. 11.) Septation of the spores, which was a characteristic of the spores of the brand-canker fungus, occurred only rarely.

PATHOGENICITY OF THE ORGANISMS

INOCULATION EXPERIMENTS

Rose cuttings grown in pots of sterilized sand were inoculated as in the experiments conducted with the brand-canker fungus. In all cases cankers were produced on both the cuttings inoculated in the axil of a bud and those inoculated through wounds. The fungus was reisolated from the diseased areas. The pycnidia produced on the cankers showed both simple and complex structures.

Cuttings of red-raspberry canes were similarly inoculated, and cankers resulted. The pycnidia differed from those produced on the rose in that they showed a tendency to be single and unilocular rather than complex plurilocular.

Inoculations of leaves in moist chambers proved unsuccessful. Since a collection of specimens of hips from hybrid plants of *Rosa rugosa* showed an infection by a fungus resembling *Coniothyrium fuckelii*, a number of healthy hips were inoculated in moist chambers with the spores of the fungi isolated from the graft cankers and the stem cankers. Pycnidia and spores were readily produced on the inoculated hips in all cases. (Fig. 12.)

PENETRATION OF THE HOST

From the results of the inoculations on the rose cuttings it is evident that infection may take place through either dormant buds or wounds. An examination of many specimens of stem canker in



FIGURE 11.—Germinating spores of the graft-canker fungus. $\times 1,300$

nature leads to the conclusion that the germ tubes are capable of entering the tissue even if no wounds or buds are present. Such infection, however, seems to be extremely rare. The diseased areas usually occur around wounds such as those caused by the breaking or pruning of stems, by the rubbing of the prickles of one stem against the bark of another, by the accidental removal of the prickles, or by insects. Rarely does one find a pruned stem which does not have a few pycnidia on the cut end at the inner margin of the bark. The same condition is very frequently found on rose cuttings, particularly those made from a plant affected with stem canker. Graft cankers also appear to result from infection through wounds or through the callus at the point of union of stock and scion. In the present study it was found that the fungus may also infect through dormant buds.

CONCLUSIONS

It is evident from this study of the morphology and physiology of the fungi isolated from the three types of cankers that the brand-canker fungus is a different species of *Coniothyrium* from that causing the so-called stem cankers and graft cankers. This corroborates the statements made by Laubert (10) and by Jenkins and Martin (8), who attributed the disease to *Coniothyrium wernsdorffiae*. In the case of the fungi isolated from stem canker and graft canker there was no evidence of any morphological or physiological differences between them. Since the fungus obtained in cultures from the stem cankers was similar to that produced in cultures from spores of *Leptosphaeria coniothyrium*, the writer felt justified in considering it to be *C. fuckelii*. The results of the present study would seem to indicate, therefore, that the graft cankers were produced by the same fungus that caused the stem cankers. The graft-canker fungus, moreover, resembled that collected and identified by Vogel (22) as *C. rosarum* in so far as its morphology under natural conditions is concerned. Unfortunately, type specimens of *C. rosarum* were not available for examination. Regardless of this fact, however, the conclusion may be drawn that the two types of cankers are caused by one and the same fungus, which resembles the imperfect stage of *L. coniothyrium* described by Saccardo as *C. fuckelii*.



FIGURE 12.—A rose hip infected with the stem-canker fungus, showing the concentric formation of the pycnidia on the infected spot

SUMMARY

Three diseases attributed to species of *Coniothyrium* have been described as occurring upon the genus *Rosa* in the United States. Because of the similarity of the symptoms of the diseases, there has been considerable doubt as to the identity of the causal fungi. The present study was undertaken to determine what morphological and physiological characters might be of value in diagnosing the diseases.

The fungus isolated from the so-called brand-canker disease may be distinguished from other species of *Coniothyrium* on rose by the pronounced buffer tissue and papilla as well as the long ostiolar canal of its pycnidia, the size of its spores, and the grayish color of its mycelium in culture. Spore germination and the rate of growth in culture were relatively slow. The spores of the fungus were produced by budding from the layer of cells lining the pycnidial cavity. Inoculation experiments showed that the fungus was capable of infecting rose and red-raspberry cuttings through dormant buds and

through wounds. Inoculations of rose leaves and hips were not successful. The fungus is designated as *Coniothyrium wernsdorffiae* Laub.

No morphological or physiological differences could be detected between the fungi isolated from the so-called stem cankers and graft cankers. The pycnidia varied from simple uniloculate to complex pluriloculate structures and developed an inconspicuous buffer tissue and papillate ostiole. The mycelium in culture was white or light gray. The spores were produced by budding, as in *Coniothyrium wernsdorffiae*. Rose and red-raspberry cuttings were successfully inoculated through dormant buds and through wounds, with the production of cankers. Rose hips became readily infected following inoculation, but no growth occurred on rose leaves. The results of this study seem to indicate that the stem cankers and graft cankers were caused by the same species of *Coniothyrium*, which is designated as *C. fuckelii* Sacc.

LITERATURE CITED

- (1) ARCHER, W. A.
1926. MORPHOLOGICAL CHARACTERS OF SOME SPHAEROPSIDALES IN CULTURE, WITH REFERENCE TO CLASSIFICATION. *Ann. Mycol.* 24: 1-84, illus.
- (2) COOKE, M. C., and HARKNESS, W. H.
1883-84. NEW CALIFORNIAN FUNGI. *Grevillea* 12: 83-84, 92-97.
- (3) DODGE, B. O.
1923. ORIGIN OF THE CENTRAL AND OSTIOLAR CAVITIES IN PYCNIDIA OF CERTAIN FUNGUS PARASITES OF FRUITS. *Jour. Agr. Research* 23: 743-750, illus.
- (4) GÜSSOW, H. T.
1909. PARASITIC ROSE CANKER. A NEW DISEASE IN ROSES. *Jour. Roy. Hort. Soc.* 34: 222-230, illus.
- (5) HÖHNEL, F. VON.
1923. SYSTEM DER FUNGI IMPERFECTI FÜCKEL. *In* Falck, *Mykologische Untersuchungen und Berichte* 3(9): 301-369.
- (6) HOWITT, J. E.
1925. SOME NOTES ON DISEASES NEW TO ONTARIO. (Abstract) *Phytopathology* 15: 300.
- (7) JENKINS, A. E.
1927. BROWN CANKER OF THE ROSE. *Amer. Rose Ann.* 1927: 161-183, illus. [Footnote on Symptoms of Brand Canker by C. Westcott, p. 166.]
- (8) ——— and MARTIN, G. H.
1926. BRAND CANKER OF ROSE APPEARS IN AMERICA. *U. S. Dept. Agr. Off. Rec.* 5(25): 3.
- (9) KOCK, G.
1905. EIN FÜR ÖSTERREICH NEUER ROSENSCHÄDLING. *Ztschr. Landw. Versuchsw. Österr.* 8: 660-666, illus.
- (10) LAUBERT, R.
1905. EINE NEUE ROSENKRANKHEIT, VERURSACHT DURCH DEN PILZ CONIOTHYRIUM WERNSDORFFIAE. *Arb. K. Biol. Anst. Land u. Forstw.* 4: 458-460, illus.
- (11) ———
1907. DIE VERBREITUNG UND BEDEUTUNG DER BRANDFLECKENKRANKHEIT DER ROSEN UND RATSCHLÄGE ZUR BEKÄMPFUNG DER KRANKHEIT. (EINE NEUE PFLANZENPATHOLOGISCHE UNTERSUCHUNG.). *Gartenwelt* 11: 332-334, 357-358, 378-380, illus.
- (12) LEONIAN, L. H.
1924. A STUDY OF THE FACTORS PROMOTING PYCNIDIA-FORMATION IN SOME SPHAEROPSIDALES. *Amer. Jour. Bot.* 11: 19-50.
- (13) LIND, J.
1913. DANISH FUNGI AS REPRESENTED IN THE HERBARIUM OF E. ROSTRUP. 648 p., illus. Copenhagen.

-
- (14) MARTIN, G. H.
1929. POLYMORPHISM OF LEPTOSPHAERIA CONIOTHYRIUM (FCKL.) SACC. *Phytopathology* 19: 879.
- (15) MASSEE, G.
1915. DISEASES OF CULTIVATED PLANTS AND TREES. Ed. 2, 602 p., illus. New York.
- (16) O'GARA, P. J.
1911. PARASITISM OF CONIOTHYRIUM FUECKELII. *Phytopathology* 1: 100-102, illus.
- (17) PETRAK, F.
1924. MYKOLOGISCHE NOTIZEN VII. *Ann. Mycol.* 22: 1-182.
- (18) POETEREN, N. VAN.
1926. VERSLAG OVER DE WERKZAAMHEDEN VAN DEN PLANTENZIEKTENKUNDIGEN DIENST IN HET JAAR 1925. Verslag. en Meded. Plantenziektenkund. Dienst Wageningen 44, 124 p., illus. [Original not seen. (Abstract) *Rev. Appl. Mycol.* 6: 462. 1927.]
- (19) SACCARDO, P.
1884. SYLLOGE FUNGORUM, v. 3, 860 p. Patavii.
- (20) STEWART, F. C.
1910. NOTES ON NEW YORK PLANT DISEASES, I. N. Y. State Agr. Expt. Sta. Bul. 328, p. [305]-404, illus.
- (21) THOMÉ, O. W.
1921. FLORA VON DEUTSCHLAND, DEUTSCH-ÖSTERREICH, UND DER SCHWEIZ. Bd. 3, t. 4, Abt. 1, 253 p., illus. Berlin.
- (22) VOGEL, I. H.
1919. A ROSE GRAFT DISEASE. *Phytopathology* 9: [403]-412, illus.
- (23) WATERMAN, A. M.
1928. ROSE DISEASES: THEIR CAUSES AND CONTROL. U. S. Dept. Agr. Farmers' Bul. 1547, 20 p., illus.
- (24) WILSON, M., and HAHN, G. G.
1928. THE IDENTITY OF PHOMA PITYA SACC., PHOMA ABIETINA HART. AND THEIR RELATION TO PHOMOPSIS PSEUDOTSUGAE WILSON. *Brit. Mycol. Soc. Trans.* 13: 261-268, illus.

INHERITANCE OF CERTAIN SEED-COAT COLORS IN SOYBEANS¹

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INTRODUCTION

Since varieties and strains of soybeans (*Soja max*) are largely identified by the color of their seed coats, it is of no small consequence to the plant breeder to learn as much as possible about the inheritance of the different color types and patterns. Some work on this problem had been published by different investigators prior to 1923 when the writer took up this study. Since then other investigators have published their findings on seed-coat inheritance in soybeans. The results obtained by the various workers have, however, been rather conflicting and in some cases have been based on few data largely obtained in the F_2 generation. It seems, then, that additional data on the inheritance of seed-coat color in soybeans should be of value in helping to clear up the problem.

In taking up this study the writer originally planned to use several varieties, to make a large number of crosses between them, and to study progenies from these crosses through the F_2 and F_3 generations, but owing to the extreme difficulty encountered in hybridizing soybeans the original plans were to a considerable extent abandoned. However, during the progress of the work the writer has been able to make a rather extensive study of a few crosses which involve some interesting genetic relationships between certain pigment colors in the seed coat. Most of these relationships have been reported by previous workers, and although their findings and symbols are somewhat different, their work contributes much to the solution of the problem. The results of all previous genetic work with soybeans have been summarized by Owen (5)⁴ and need not be taken up here. Closely related work will, however, be discussed whenever it has a direct bearing on the problem in hand.

MATERIALS AND METHODS

In this study only varieties of soybeans with yellow, black, and brown pigments in their seed coats have been used. No crosses involving green seed-coat pigments were available. Most of the data used in this study were obtained from progenies of F_1 seeds of crosses turned over to the writer in the spring of 1923 by John B. Wentz, of the department of farm crops and soils, Iowa State College.

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² The writer acknowledges the many suggestions and untiring assistance rendered by Dr. John B. Wentz in planning and bringing this problem to completion. He also wishes to extend thanks to Prof. H. D. Hughes and F. S. Wilkins for freely lending equipment necessary to carry on the work. Dr. E. W. Lindstrom and Prof. W. V. Lambert have given helpful suggestions in the preparation of the thesis.

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⁴ Reference is made by number (italic) to "Literature cited," p. 854.

Since that time the writer has added two crosses and two natural hybrids.

In this investigation the progenies of 13 different crosses involving 8 different combinations of varieties and 2 natural hybrids have been studied. During the course of this work the writer has been able to observe the genetic behavior of most of the crosses through the F_3 generation. One family in cross No. 5 has been grown through the F_4 generation.

In order to avoid as far as possible any mixing or contamination, seeds from the F_1 and F_2 plants were harvested and threshed by hand. At the time of harvesting, the seeds from each plant were put into separate envelopes and the envelopes numbered according to the row and plant number. All plants of progenies segregating for pubescence color were tagged several weeks before harvesting in order that pubescence and seed-coat relationships could be studied. The F_3 plants were pulled by hand and tied into bundles. Individual plants were threshed with a small threshing machine and the seeds from each plant put into a numbered envelope.

INHERITANCE OF HILUM COLOR

Woodworth (?) reported data on the inheritance of black and brown hilum color. Black hilum color was reported as being dominant to brown. To explain his data, it was necessary to assume two factors, *B* and *H*. When both factors were present in the zygote the hilum color was black. If either *B* or *H* was absent, the hilum color was brown. Thus a 9 to 7 interaction of factors was established. Individuals without either *B* or *H*, but containing their recessive allelomorphs, *b* and *h*, could not be distinguished from those having only one of the dominant factors. In all other cases thus far reported the inheritance of hilum color has been explained by assuming a single-factor difference.

In the present studies two crosses, No. 113 and No. 527, which involve different hilum colors, were obtained. Data for these crosses are presented in Table 1.

TABLE 1.— F_2 progenies from crosses between varieties of soybeans having brown and black hilums, Ito San \times Manchu

Cross No.	Black hilum	Brown hilum	Deviation 3:1	Deviation P. E.
113-1.....	56	17	1.25	0.50
113-2.....	61	16	3.25	1.27
527.....	9	4	.75	.71
Total.....	126	37	3.75	1.00

The male parent was a selection from the Ito San variety and the female parent a selection from the Manchu variety. Both parents for these crosses have yellow seed coats, but the male parent has a brown hilum and the female a black hilum. Two F_1 plants were obtained from cross No. 113 and one F_1 plant from cross No. 527. Pure lines of both parents bore seeds which were extremely mottled, the color of the mottling always being the same as the color of the

hilum. Seeds borne on the F_1 plants were yellow with black hilums and black mottling over the sides. In the F_2 generation, plants bearing seeds with black hilums and plants bearing seeds with brown hilums appeared in approximately a 3:1 ratio. From the 2 F_1 plants in cross No. 113, 117 F_2 plants bearing seeds with black hilums and 33 plants bearing seeds with brown hilums were obtained. The deviation from a 3:1 ratio in this cross was 4.50 ± 3.58 . Only 13 F_2 plants were obtained from cross No. 527. Of these, 9 bore yellow seeds with black hilums and 4 yellow seeds with brown hilums. In this cross the deviation from a 3:1 ratio was 0.75 ± 1.05 .

When both crosses are considered together, 126 of the F_2 plants bore yellow seeds with black hilums and 37 yellow seeds with brown hilums. The deviation from a 3:1 ratio with all plants considered is 3.75 ± 3.75 . This difference can well be attributed to chance, and one factor pair suffices to explain the data.

In these crosses pigment in the hilum is apparently due to the same factors as in the seed coat, and since the author is using the factors adopted by Owen (5) for seed-coat color, the factor pair R_{17} is used instead of Bb used by Woodworth.

INHERITANCE OF SEED-COAT COLORS

In these studies crosses have been made between varieties which involve different color combinations of the seed coat. In order to present the results more clearly it is thought advisable to give the data for these different color combinations separately.

CROSSES INVOLVING LIGHT-BROWN AND REDDISH-BROWN SEED COATS

Nagai (1) has reported results of crosses between light-brown and reddish-brown varieties of soybeans. His data show that reddish-brown is recessive to the lighter brown color. A single-factor pair, O_o , is responsible for the difference.

The writer has been able to gather considerable data on the inheritance of these two colors from reciprocal crosses between the Ogemaw and Soysota varieties. The Ogemaw variety has a reddish-brown seed coat with a more or less dull appearance. The Soysota variety, on the other hand, has a light-brown seed coat with a more or less glossy appearance. F_1 seeds from these crosses, regardless of the way the crosses were made, were more or less intermediate between the two parents in color. However, they more nearly approach the shade of the lighter-colored parent. The F_2 plants appeared to show the three classes—light, intermediate, and reddish-brown colors—suggesting incomplete dominance. The F_2 plants were classified into the three classes, although considerable difficulty was experienced in separating the light-brown from the intermediate class. No difficulty was experienced, however, in separating the reddish-brown class.

A larger number of the F_2 plants were tested in the F_3 generation. The seed-coat colors observed in the F_3 generation showed that many of the F_2 plants classified as light brown were heterozygous; also that many of the F_2 plants classified as intermediate failed to segregate as expected for the reddish-brown color. Thus, the F_3 results showed the fallacy of trying to separate the light-brown from the intermediate class. With a reclassification—that is, grouping the light and intermediate classes together—there were obtained from all of the crosses

between these two varieties 472 F_2 plants with light-brown seeds and 149 with reddish-brown seeds. (Table 2.) The deviation from the expected 3:1 ratio was 6.25 ± 7.28 , confirming a single-factor difference between the light-brown and reddish-brown pigment colors.

The symbol r_1 has been used by Owen to designate the factor for the light-brown pigment of the Soysota variety, and o has been used by Nagai to designate the factor for reddish-brown pigment of the Ogemaw variety. Following the nomenclature of both of these authors, r_1 is used as the factor for light brown and r_1^o the factor for reddish brown. Data for the F_2 plants are presented in Table 2.

TABLE 2.— F_2 progenies from reciprocal crosses between varieties of soybeans having light-brown and reddish-brown seed coats

Cross	Light brown	Reddish brown	Deviation 3:1	Deviation P. E.
Soysota \times Ogemaw:				
No. 50-1	61	22	1.25	0.47
No. 50-2	63	22	.75	.29
Ogemaw \times Soysota:				
No. 64-1	62	23	1.75	.66
No. 64-2	65	25	2.50	.90
No. 65-1	51	15	1.50	.63
No. 65-2	63	11	7.50	2.99
No. 73-1	54	14	3.00	1.24
No. 73-2	53	17	.50	.20
Total	472	149	6.25	.86

TABLE 3.—Assumed genotypes together with breeding behavior of F_2 plants when tested in the F_3 generation

[Cross No. 50 Soysota ($ii r_1 r_1 R_2 R_2$) \times Ogemaw ($ii r_1^o r_1^o R_3 R_3$)

Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a	
	Light brown	Reddish brown		Light brown	Reddish brown		Light brown	Reddish brown
$ii r_1 r_1 R_2 R_2$:			$ii r_1^o r_1^o R_2 R_2$ —			$ii r_1 r_1^o R_2 R_2$ —		
1-4	14	—	Contd.			Contd.		
1-5	40	—	1-40	42	1-33	1-33	20	8
1-6	40	—	1-44	36	1-34	1-34	38	7
1-8	53	—	1-48	40	1-35	1-35	29	7
1-11	49	—	1-49	12	1-36	1-36	29	12
1-12	31	—	1-50	39	1-38	1-38	21	8
1-15	21	—	1-54	29	1-41	1-41	27	4
1-16	38	—	1-55	29	1-42	1-42	37	12
1-21	44	—	$ii r_1 r_1^o R_2 R_2$:			1-45	34	5
1-30	36	—	1-1	35	1-46	1-46	22	6
1-37	14	—	1-2	42	1-51	1-51	27	5
1-47	34	—	1-3	33	1-52	1-52	13	2
1-58	52	—	1-7	9	1-53	1-53	39	12
1-61	45	—	1-13	30	1-56	1-56	29	9
1-88	23	—	1-14	28	1-57	1-57	32	6
$ii r_1 r_1^o R_2 R_2$:			1-18	33	1-59	1-59	11	2
1-9	42	—	1-23	16	1-60	1-60	30	15
1-20	51	—	1-26	38	1-86	1-86	28	12
1-22	50	—	1-27	21	1-87	1-87	23	12
1-24	8	—	1-28	27				
1-32	52	—	1-29	23				
1-39	47	—	1-31	30				
					Total		854	273

^a Deviation
P. E. = 0.89.

From cross No. 50 there were 59 F_2 plants tested; from cross No. 64, 97; and from cross No. 65, 147, making a total of 303 F_2 plants tested in the F_3 generation. Of these, 60 were classified as having reddish-brown seed coats and in the F_3 generation bred true. The remaining 243 were classified as having light-brown seed coats. On the basis of a single-factor hypothesis, 81 of the 243 F_2 plants classed as having light-brown seed coats should breed true and 162 should segregate again for 3 light-brown to 1 reddish-brown. The actual counts were 81 breeding true and 162 segregating, an exact fit. From the 81 F_2 plants showing no segregation 2,771 F_3 plants were grown. From the 162 F_2 plants which segregated, 6,192 F_3 plants were obtained. Of these, 4,688 were light brown and 1,504 were reddish brown. The deviation from the expected 3:1 ratio is 44.00 ± 20.00 . The F_3 data for the above crosses are presented in Tables 3, 4, and 5.

TABLE 4.—Assumed genotype together with breeding behavior of F_2 plants tested in the F_3 generation

[Cross No. 64 Ogemaw ($ii\ r_1r_1 \cdot R_2R_2$) \times Soysota ($ii\ r_1r_1\ R_2R_2$)]

Genotypes and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		Genotypes and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		Genotypes and F_2 plant No.	Phenotypes observed in the F_3 generation ^a	
	Light brown	Reddish brown		Light brown	Reddish brown		Light brown	Reddish brown
$ii\ r_1r_1\ R_2R_2$			$ii\ r_1r_1 \cdot R_2R_2$ —			$ii\ r_1r_1 \cdot R_2R_2$ —		
1-8-----	25	-----	Contd.			Contd.		
1-10-----	49	-----	2-60-----	-----	44	2-35-----	39	11
1-13-----	8	-----	2-61-----	-----	29	2-36-----	37	11
1-17-----	6	-----	2-66-----	-----	41	2-37-----	34	15
2-18-----	25	-----	2-65-----	-----	53	2-38-----	44	7
2-20-----	37	-----	2-68-----	-----	42	2-46-----	19	6
2-21-----	53	-----	2-82-----	-----	44	2-47-----	32	10
2-28-----	43	-----	2-89-----	-----	51	2-49-----	29	11
2-34-----	42	-----	2-91-----	-----	2	2-52-----	26	9
2-39-----	43	-----	2-94-----	-----	50	2-54-----	41	14
2-45-----	18	-----	2-95-----	-----	43	2-55-----	38	10
2-53-----	46	-----	$ii\ r_1r_1 \cdot R_2R_2$			2-57-----	32	20
2-59-----	5	-----	1-1-----	21	11	2-58-----	21	3
2-61-----	45	-----	1-2-----	39	13	2-60-----	22	14
2-62-----	39	-----	1-3-----	40	7	2-63-----	32	16
2-64-----	45	-----	1-4-----	28	6	2-67-----	39	9
2-66-----	40	-----	1-6-----	39	8	2-69-----	21	8
2-73-----	47	-----	1-7-----	24	9	2-70-----	24	4
2-77-----	43	-----	1-12-----	42	16	2-71-----	46	5
2-81-----	33	-----	1-14-----	23	3	2-72-----	23	16
2-83-----	6	-----	1-18-----	30	15	2-74-----	37	11
2-88-----	30	-----	2-12-----	11	2	2-75-----	37	9
2-90-----	33	-----	2-13-----	32	8	2-76-----	5	6
$ii\ r_1r_1 \cdot R_2R_2$			2-14-----	33	7	2-78-----	31	10
1-6-----		38	2-15-----	19	6	2-79-----	28	4
1-11-----		33	2-16-----	51	9	2-80-----	33	14
1-15-----		49	2-19-----	34	10	2-84-----	31	7
1-16-----		48	2-22-----	36	12	2-86-----	38	9
2-17-----		34	2-23-----	23	8	2-87-----	34	9
2-29-----		47	2-24-----	34	8	2-92-----	25	8
2-33-----		38	2-25-----	23	10	2-93-----	24	8
2-40-----		48	2-27-----	17	7			
2-42-----		35	2-30-----	31	13	Total-----	1,575	497
2-44-----		28	2-31-----	33	10			
2-48-----		47	2-32-----	10	5			

^a Deviation = 1.58.
P. E.

TABLE 5.—Assumed genotypes together with breeding behavior of F_2 plants when tested in the F_3 generation[Cross No. 65 Ogemaw ($ii r_1^o r_1^o R_2 R_2$) \times Soysota ($ii r_1 r_1 R_2 R_2$)

F_2 genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		F_2 genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		F_2 genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a	
	Light brown	Reddish brown		Light brown	Reddish brown		Light brown	Reddish brown
<i>ii r_1 r_1 R_2 R_2</i> :			<i>ii r_1^o r_1^o R_2 R_2</i> — Contd.			<i>ii r_1 r_1^o R_2 R_2</i> — Contd.		
1-5.....	46	-----	1-44.....	-----	40	1-65.....	37	5
1-8.....	45	-----	1-49.....	-----	24	1-67.....	23	15
1-9.....	30	-----	1-55.....	-----	20	1-68.....	27	21
1-17.....	38	-----	1-56.....	-----	41	1-70.....	22	6
1-22.....	42	-----	1-58.....	-----	22	1-73.....	31	14
1-25.....	47	-----	1-59.....	-----	18	1-74.....	35	11
1-28.....	48	-----	1-61.....	-----	36	1-75.....	30	10
1-31.....	37	-----	1-63.....	-----	43	1-76.....	26	14
1-34.....	44	-----	1-66.....	-----	46	1-79.....	14	6
1-41.....	31	-----	1-77.....	-----	29	1-80.....	28	5
1-46.....	46	-----	1-78.....	-----	12	2-1.....	23	10
1-50.....	44	-----	2-21.....	-----	39	2-3.....	34	9
1-51.....	20	-----	2-22.....	-----	39	2-5.....	36	12
1-54.....	34	-----	2-23.....	-----	20	2-9.....	37	12
1-60.....	26	-----	2-38.....	-----	40	2-10.....	31	8
1-62.....	23	-----	2-43.....	-----	36	2-12.....	32	13
1-71.....	22	-----	2-65.....	-----	3	2-15.....	24	8
1-72.....	24	-----	2-67.....	-----	31	2-17.....	32	7
2-2.....	47	-----	2-70.....	-----	34	2-18.....	34	8
2-4.....	28	-----	<i>ii r_1 r_1^o R_2 R_2</i> :			2-20.....	34	11
2-6.....	23	-----	1-1.....	31	9	2-25.....	29	10
2-7.....	52	-----	1-2.....	38	8	2-26.....	42	9
2-11.....	17	-----	1-3.....	38	9	2-27.....	28	8
2-14.....	22	-----	1-4.....	31	17	2-29.....	23	7
2-16.....	48	-----	1-6.....	31	14	2-33.....	28	12
2-19.....	13	-----	1-7.....	43	11	2-34.....	37	11
2-23.....	25	-----	1-11.....	35	6	2-35.....	29	17
2-24.....	3	-----	1-13.....	29	10	2-36.....	34	24
2-32.....	21	-----	1-14.....	31	14	2-39.....	11	3
2-37.....	46	-----	1-18.....	5	8	2-41.....	28	15
2-40.....	33	-----	1-19.....	30	9	2-43.....	25	4
2-45.....	35	-----	1-20.....	38	11	2-44.....	41	11
2-48.....	44	-----	1-24.....	29	10	2-46.....	15	4
2-50.....	25	-----	1-29.....	33	9	2-47.....	28	9
2-51.....	44	-----	1-30.....	33	12	2-49.....	36	11
2-56.....	39	-----	1-35.....	20	8	2-62.....	31	11
2-58.....	34	-----	1-36.....	22	8	2-63.....	35	8
2-68.....	34	-----	1-37.....	28	6	2-64.....	35	3
2-72.....	48	-----	1-38.....	33	7	2-65.....	30	15
2-73.....	30	-----	1-39.....	31	10	2-61.....	12	3
2-74.....	49	-----	1-40.....	25	4	2-62.....	12	3
2-75.....	23	-----	1-42.....	23	11	2-63.....	31	11
2-76.....	46	-----	1-43.....	31	10	2-64.....	34	5
<i>ii r_1^o r_1^o R_2 R_2</i> :			1-45.....	34	11	2-66.....	34	7
1-10.....	42	-----	1-47.....	28	10	2-69.....	13	5
1-12.....	45	-----	1-48.....	33	4	2-71.....	30	6
1-15.....	32	-----	1-52.....	9	4	2-77.....	33	16
1-16.....	19	-----	1-53.....	7	3	2-78.....	47	8
1-21.....	40	-----	1-57.....	35	9			
1-26.....	49	-----	1-64.....	26	11			
1-33.....	40	-----				Total.....	2,259	734

^a Deviation
P. E. = 0.89.

CROSS INVOLVING VARIETIES WITH BLACK AND BROWN SEED COATS

Piper and Morse (6) reported black pigment to be dominant to brown. A single-factor difference explained their data. Owen (5) reported on the inheritance of black and brown seed-coat colors. In one case his data conform to the findings of Piper and Morse. In this cross black was found to be fully dominant to brown, and the assumption of a single-factor difference sufficed to explain his data.

In the second case the behavior was found to be quite different. Data taken from a natural hybrid showed incomplete dominance of black over brown. An intermediate class which was black and speckled with brown was observed. Further breeding showed these intermediate classes to be heterozygous.

The writer has been able to obtain but one cross involving these two colors, that is, cross No. 502 (Wisconsin Black \times Soysota). Seeds borne on the F_1 plant were fully black. Only 13 F_2 plants were obtained. Of these, 9 were black and 4 were brown. The deviation from the expectant 3 : 1 ratio was 0.75 ± 1.05 . The results can, therefore, be explained on the basis of a single pair of factors, black being dominant over brown. The factors R_1r_1 proposed by Owen are used to designate this difference.

CROSSES INVOLVING VARIETIES HAVING YELLOW AND BLACK SEED COATS

Data on three crosses between varieties having yellow and black seed coats have been obtained. Cross No. 206 (Manchu \times Wisconsin Black) and cross No. 407 (Manchu \times Wilson) are very similar and may be discussed together. In these two crosses the female parents are from the same variety, Manchu. Pure lines from the female parent of each cross were grown and as far as seed-coat color is concerned could not be distinguished, the seed-coat color being in each case yellow, mottled with black. The male parent in each cross bore black seeds. There was, however, considerable difference in their habit of growth. The Wisconsin Black parent stood erect and was more or less bushy while the parent from the Wilson variety was of a reclining type and showed a tendency to be more or less viny.

The F_1 seeds from both varieties were yellow, mottled with black, similar to the seeds of the pure-line female parents. In the F_2 generation, from cross No. 206, 45 plants were obtained. Of these, 34 were yellow mottled with black, though in varying degrees, and 11 were self black. On the basis of a 3 : 1 ratio, the deviation from the expected was 0.25 ± 1.96 . From cross No. 407, 122 plants bearing seeds which were yellow mottled with black and 37 plants bearing seeds which were self black were obtained in the F_2 generation. In this cross the deviation from the expected 3 : 1 ratio was 2.75 ± 3.68 . When both crosses were considered together, there were 204 F_2 plants. Of these, 156 bore seeds which were yellow mottled with black and 48 seeds which were self black. The deviation from the expected 3 : 1 ratio was 3.00 ± 4.17 . The F_2 data for these crosses are presented in Table 8. Data for the breeding behavior of the F_2 plants are shown in Tables 6 and 7. A single pair of factors will explain the difference, but in this case pigment factors are not involved, since all plants bearing yellow seeds contained black pigment in the hilum. Black pigment also occurred in the seed coat of all mottled beans. The difference then must be in another factor which inhibits or partially inhibits yellow from most of the seed coat. Owen (6) has used two such inhibiting factors I^h and I^i which form a multiple allelomorphic series with i to designate this difference. According to his hypothesis a plant homozygous or heterozygous for either I^h or I^i would bear yellow seeds, and a plant homozygous for their recessive allelomorphic, i , would bear self-colored seeds. In these studies the writer was unable to distinguish between I^h and I^i . Therefore, I is used as the factor which inhibits pigment color and the factor pair involved is assumed to be $I\bar{i}$.

TABLE 6.—Assumed genotypes together with breeding behavior of F_2 plants when tested in the F_3 generation[Cross No. 200 Manchu ($II R_1R_1 R_2R_2$) \times Wisconsin Black ($ii R_1R_1 R_2R_2$)]

Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a	
	Mottled, yellow black	Self-colored black		Mottled, yellow black	Self-colored black		Mottled, yellow black	Self-colored black
$II R_1R_1 R_2R_2$:			$ii R_1R_1 R_2R_2$ —			$II R_1R_1 R_2R_2$ —		
1-3.....	2	-----	Continued.			Continued.		
1-6.....	15	-----	1-20.....		32	1-27.....	28	4
1-7.....	15	-----	1-21.....		27	1-31.....	9	6
1-10.....	9	-----	1-22.....		25	1-33.....	25	3
1-12.....	1	-----	1-23.....		31	1-35.....	2	1
1-13.....	4	-----	1-32.....		28	1-36.....	15	3
1-19.....	22	-----	1-45.....		19	1-37.....	12	9
1-23.....	22	-----	1-43.....		32	1-38.....	18	4
1-26.....	15	-----	$II R_1R_1 R_2R_2$:			1-39.....	10	9
1-29.....	20	-----	1-1.....	20	6	1-41.....	2	1
1-34.....	15	-----	1-3.....	10	4	1-42.....	18	3
1-50.....	34	-----	1-9.....	8	5	1-43.....	53	10
$ii R_1R_1 R_2R_2$:			1-15.....	21	5	1-44.....	14	8
1-2.....		16	1-17.....	10	3	1-45.....	24	3
1-5.....		11	1-18.....	17	2	1-46.....	12	4
1-11.....		28	1-24.....	7	4			
1-16.....		19	1-25.....	22	4	Total.....	337	101

^a Deviation
P. E. = 1.39.TABLE 7.—Assumed genotypes together with breeding behavior of F_2 plants when tested in the F_3 generation[Cross No. 407 Manchu ($II R_1R_1 R_2R_2$) \times Wilson ($ii R_1R_1 R_2R_2$)]

Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a		Genotype and F_2 plant No.	Phenotypes observed in the F_3 generation ^a	
	Mottled, yellow black	Self-colored black		Mottled, yellow black	Self-colored black		Mottled, yellow black	Self-colored black
$II R_1R_1 R_2R_2$:			$ii R_1R_1 R_2R_2$ —			$II R_1R_1 R_2R_2$ —		
15.....	22	-----	Continued.			Continued.		
20.....	21	-----	22.....		34	38.....	25	13
21.....	34	-----	24.....		13	42.....	17	6
23.....	19	-----	26.....		20	43.....	30	6
29.....	29	-----	31.....		37	46.....	14	7
39.....	17	-----	32.....		19	52.....	22	3
41.....	37	-----	35.....		38	53.....	33	3
45.....	17	-----	44.....		34	55.....	13	2
48.....	41	-----	49.....		33	57.....	15	4
50.....	46	-----	62.....		36	60.....	29	3
51.....	30	-----	64.....		17	61.....	32	12
54.....	31	-----	73.....		10	63.....	23	6
58.....	29	-----	80.....		27	66.....	30	8
59.....	26	-----	89.....		5	67.....	17	5
65.....	37	-----	99.....		28	69.....	22	13
69.....	39	-----	101.....		38	71.....	24	10
70.....	36	-----	102.....		35	74.....	25	6
72.....	24	-----	$II R_1R_1 R_2R_2$:			75.....	28	5
73.....	33	-----	1.....			76.....	30	10
79.....	33	-----	2.....	29	10	77.....	31	8
81.....	25	-----	4.....	16	14	82.....	31	11
87.....	36	-----	5.....	30	9	83.....	30	7
88.....	35	-----	8.....	33	9	84.....	25	7
90.....	35	-----	9.....	26	10	85.....	28	8
93.....	34	-----	13.....	20	12	91.....	18	9
97.....	36	-----	14.....	25	7	92.....	16	14
$ii R_1R_1 R_2R_2$:			16.....	19	10	94.....	24	5
3.....		23	18.....	25	7	95.....	16	6
6.....		25	25.....	27	14	96.....	9	6
7.....		27	26.....	20	10	98.....	25	9
10.....		20	27.....	20	10	100.....	25	2
11.....		37	28.....	24	6	103.....	26	10
12.....		24	30.....	11	9	Total.....	1,122	387
17.....		36	33.....	23	7			
19.....		29	34.....	21	11			
			36.....	20	3			

^a Deviation
P. E. = 0.86.

In cross No. 5 (Wisconsin Black \times Mandarin) quite different results were obtained. Owen (5) has reported data from combinations very similar to this cross. In certain of his crosses the Mandarin variety but not the Wisconsin Black was used. Data presented by Owen showed that three factors were involved in his crosses. The phenotypes which are being reported in this study were also reported by Owen, which indicates that the crosses were genetically alike. The female parent of this cross had tawny pubescence and bore self-colored black seeds. The male parent had gray pubescence and bore yellow seeds, mottled with a buff pigment. The F_1 plant had tawny pubescence and produced seeds which were yellow mottled with black. In the F_2 generation 8 different phenotypes were produced in a population of 56 plants, which suggested 3 factors to be involved. The number of plants secured in each of the phenotypes, together with the number expected, are presented in Table 8.

TABLE 8.— F_2 progenies from crosses between varieties of soybeans having yellow and black seed coats

Cross	Tawny pubescence				Gray pubescence			
	Mottled		Self-colored		Mottled		Self-colored	
	Yellow black	Yellow brown	Black	Brown	Imperfect black	Buff	Imperfect black	Buff
No. 206, Manchu \times Wisconsin Black	34	-----	11	-----	-----	-----	-----	-----
No. 407, Manchu \times Wilson	122	-----	37	-----	-----	-----	-----	-----
Total observed	156	-----	48	-----	-----	-----	-----	-----
Deviation 3:1	+3	-----	-3	-----	-----	-----	-----	-----
No. 5, Wisconsin Black \times Mandarin	24	9	6	4	6	2	4	1
Expected	23.6	7.9	7.9	2.6	7.9	2.6	2.6	.9

$$\chi^2=2.7. \quad P=0.9.$$

By classifying the progenies with yellow mottled seeds into one group and those bearing self-colored seeds into another group, the number of plants in each group is 41 and 15, respectively, which approximates a 3:1 ratio. The restriction factor I for pigment color used in the two preceding crosses can therefore be considered to restrict the pigment in this cross.

Disregarding the yellow color and classifying the F_2 progenies according to the color of pigment in their hilum or seed coat, there are 30 plants which bore seeds showing black pigment, 10 imperfect black, 13 brown, and 3 buff. With two factors involved, the expected ratio is 31.5:10.5:10.5:3.5. The numbers observed conform closely to the expected, and a dihybrid ratio is assumed.

The two factors R_1 and R_2 suggested by Owen (5) for black pigment can be used. R_1R_1 produces black pigment, R_1r_2 imperfect black, r_1R_2 brown, and r_1r_2 buff. Plants with the restriction factor I have the pigment confined to the hilum or regions around the hilum, and plants with its allelomorph, i , have self-colored seeds. By assuming that the three factors act independently of each other the appearance of the eight different phenotypes shown in Table 8 can be explained.

By referring again to Table 8 it may be noticed that the F_2 plants are also segregating for tawny and gray pubescence in approximately

a 3:1 ratio, and that all plants with black and brown pigment colors have tawny pubescence and all plants with imperfect black or buff pigments have gray pubescence. Since R_2 is also associated with black and brown pigment and its allelomorph r_2 is associated with imperfect black and buff pigments, it follows that R_2 must also be closely linked with a factor for tawny pubescence and r_2 with the recessive, a factor for gray pubescence. Woodworth (7) has designated this factor pair as Tt . Owen (5) has also used these symbols. They can, therefore, well be used to explain data obtained in these studies. Assuming that the four factors, I , R_1 , R_2 , and T (R_2 and T showing complete linkage) are involved in this cross, the F_2 data presented in Table 8 can well be explained. The values of χ^2 , 2.72, and of P , 0.91 indicate a much better fit than would ordinarily be obtained with only 56 F_2 plants, but the classification is well borne out by the breeding behavior of the F_2 plants shown in Table 9.

TABLE 9.—Assumed genotypes together with breeding behavior of F_2 plants when tested in the F_3 generation

[(Cross No. 5 Wisconsin Black ($ii R_1 R_1 R_2 T R_2 T$) \times Mandarin ($II r_1 r_1 r_2 t r_2 t$)]

Genotype and F ₂ Plant No.	Phenotypes observed in the F ₂ generation								Probability	
	Tawney pubescence				Gray pubescence					
	Mottled		Self-colored		Mottled		Self-colored			
	Yellow black	Yellow brown	Black	Brown	Yellow imperfect black	Yellow buff	Imperfect black	Buff	Devia- tion P. E.	χ ² and P
II R ₁ R ₁ R ₂ TR ₂ T:										
8.....	96									
51.....	61									
61.....	64									
II r ₁ r ₁ R ₂ TR ₂ T:										
38.....		93								
II r ₁ r ₁ R ₂ TR ₂ T:										
30.....				30						
60.....				57						
II R ₁ R ₁ r ₂ tr ₂ t:										
33.....					35					
II r ₁ r ₁ r ₂ tr ₂ t:										
58.....						31				
II R ₁ R ₁ r ₂ tr ₂ t:										
9.....							24			
49.....							25			
62.....							43			
r ₁ r ₁ r ₂ tr ₂ t:										
47.....								25		
II R ₁ r ₁ R ₂ TR ₂ T:										
5.....	36	16								
25.....	21	12								
53.....	20	10								
Total.....	77	38							2.96	
II R ₁ R ₁ R ₂ TR ₂ T:										
19.....	70		20						.90	
II R ₁ R ₁ R ₂ Tr ₂ t:										
27.....	14				5					
45.....					18					
Total.....	59				23				.95	
II r ₁ r ₁ R ₂ Tr ₂ t:										
3.....		25				6				
4.....		19				3				
21.....		20				7				
Total.....		64				16			1.53	

TABLE 9.—Assumed genotypes together with breeding behavior of F_2 plants when tested in the F_3 generation—Continued[(Cross No. 5 Wisconsin Black ($ii R_1 R_1 R_2 T R_2 T$) × Mandarin ($II r_1 r_1 r_2 tr_2$)]

Genotype and F ₂ Plant No.	Phenotypes observed in the F ₂ generation								Probability	
	Tawney pubescence				Gray pubescence					
	Mottled		Self-colored		Mottled		Self-colored			
	Yellow black	Yellow brown	Black	Brown	Yellow imperfect black	Yellow buff	Imperfect black	Buff	Deviation P. E.	χ ² and P
ii R ₁ R ₁ R ₂ TR ₂ T:										
6.....			21	9						
44.....			40	15						
Total.....			61	24					1.02	
ii r ₁ r ₁ R ₂ Tr ₂ t:										
32.....			13				5		.40	
ii r ₁ r ₁ R ₂ Tr ₂ t:										
13.....				81				27	0	
ii R ₁ R ₁ r ₂ tr ₂ t:						24		7	.46	
26.....										
ii r ₁ r ₁ r ₂ tr ₂ t:							42		21	2.26
54.....										
ii R ₁ r ₁ r ₂ tr ₂ t:								14	6	.76
16.....										
II R ₁ r ₁ R ₂ Tr ₂ t:										
1.....	10	3			3	1				
2.....	40	13			10	5				
7.....	51	12			15	6				
20.....	49	18			13	3				
46.....	38	6			10	4				
Total.....	188	52			51	19				2.7, .4
ii R ₁ r ₁ R ₂ TR ₂ T:										
15.....	15	5	6	1						
57.....	36	5	6	6						
Total.....	51	10	12	7						4.9, .2
ii R ₁ R ₁ R ₂ Tr ₂ t:										
48.....	12		1		4		2			
52.....	21		4		4		1			
Total.....	33		5		8		3			3.1, .4
ii r ₁ r ₁ R ₂ Tr ₂ t:										
31.....		43		12		9		8		
36.....		39		15		7		4		
37.....		29		10		14		5		
40.....		33		8		8		3		
Total.....		144		45		38		20		3.1, .4
ii R ₁ r ₁ R ₂ Tr ₂ t:										
23.....			98	21			23	7		
41.....			13	6			5	1		
45.....			33	23			7	2		
Total.....			144	50			35	10		5.1, .2
ii R ₁ r ₁ r ₂ tr ₂ t:										
17.....					39	17	9	1		
18.....					10	3	1	1		
55.....					36	15	12	2		
Total.....					85	35	22	4		6.2, .1
ii R ₁ r ₁ R ₂ Tr ₂ t:										
12.....	33	13	6	1	7	2	3	1		
35.....	30	10	9	1	4	3	4	0		
50.....	18	16	6	2	4	1	4	0		
56.....	5	1	1	1	2	1	1	0		
Total.....	86	40	22	5	17	7	12	1		15.9, .03

The interaction of the four factors can best be understood by observing the theoretical phenotypic distribution below.

27	$I R_1 R_2 T$ -----	Yellow, mottled with black—tawny pubescence.
9	$I R_1 r_2 t$ -----	Yellow, mottled with imperfect black-gray pubescence.
9	$I r_1 R_2 T$ -----	Yellow, mottled with brown—tawny pubescence.
3	$I r_1 r_2 t$ -----	Yellow, mottled with buff—gray pubescence.
9	$i R_1 R_2 T$ -----	Self-black—tawny pubescence.
3	$i R_1 r_2 t$ -----	Self-imperfect black—gray pubescence.
3	$i r_1 R_2 T$ -----	Self-brown—tawny pubescence.
1	$i r_1 r_2 t$ -----	Self-buff—gray pubescence.

Since all classes with R_2 have tawny pubescence, the question at once arises why not assume that T instead of R_2 is the complementary factor to R_1 and that it also causes the development of tawny pubescence? Woodworth (7) discussed this point and concluded that such may be the case. Owen (5) also has noticed the apparent influence T has on the seed-coat color and assumes that T is identical or completely linked with the C factor described by Nagai (1) and Nagai and Saito (2) which acts in a complementary way with R_1 to give black color. Just whether T is the only factor concerned or whether another factor, R_2 , completely linked with T is responsible for intensifying the anthocyanin pigment to black has not yet been determined. However, it seems that since varieties with black seed coat and gray pubescence are known (Piper and Morse (6)), it is more satisfactory to assume a hypothesis based on two factors which here are apparently completely linked but which may occasionally cross over to give rise to the varieties with a black seed coat and gray pubescence.

TABLE 10.—Genotype of F_2 plants as shown by their breeding behavior in the F_3 generation

[Cross No. 5 Wisconsin Black ($ii R_1 R_1 R_2 T R_2 T$) \times Mandarin ($II r_1 r_1 r_2 t r_2 t$)]

Genotype of F_2 plants				Genotype of F_2 plants			
	Observed	Calculated	$\frac{(o-c)^2}{c}$		Observed	Calculated	$\frac{(o-c)^2}{c}$
1 $II R_1 R_1 R_2 T R_2 T$ -----	3	0.797	6.089	2 $II r_1 r_1 R_2 T R_2 T$ -----	0	1.594	1.594
2 $II R_1 R_1 R_2 T r_2 t$ -----	2	1.594	.104	4 $II r_1 r_1 R_2 T r_2 t$ -----	4	3.188	.207
2 $II R_1 r_1 R_2 T R_2 T$ -----	3	1.594	1.240	1 $ii R_1 R_1 R_2 T R_2 T$ -----	0	.797	.797
2 $II R_1 R_1 R_2 T r_2 t$ -----	1	1.594	.221	2 $ii R_1 R_1 R_2 T r_2 t$ -----	1	1.594	.221
4 $II R_1 r_1 R_2 T R_2 T$ -----	5	3.188	1.030	2 $ii R_1 r_1 R_2 T R_2 T$ -----	2	1.594	.104
4 $II R_1 R_1 R_2 T r_2 t$ -----	2	3.188	.443	4 $ii R_1 r_1 R_2 T r_2 t$ -----	3	3.188	.011
4 $II R_1 r_1 R_2 T r_2 t$ -----	2	3.188	.443	1 $II r_1 r_1 r_2 t r_2 t$ -----	1	.797	.051
8 $II R_1 r_1 R_2 T R_2 T$ -----	4	6.376	.885	2 $II r_1 r_1 r_2 t r_2 t$ -----	1	1.594	.221
1 $II R_1 R_1 r_2 t r_2 t$ -----	1	.797	.051	1 $ii R_1 R_1 r_2 t r_2 t$ -----	3	.797	6.089
2 $II R_1 r_1 r_2 t r_2 t$ -----	0	1.594	1.594	2 $ii R_1 r_1 r_2 t r_2 t$ -----	1	1.594	.221
2 $II R_1 R_1 r_2 t r_2 t$ -----	1	1.594	.221	1 $ii r_1 r_1 R_2 T R_2 T$ -----	2	.797	1.816
4 $II R_1 r_1 r_2 t r_2 t$ -----	3	3.188	.011	2 $ii r_1 r_1 R_2 T r_2 t$ -----	1	1.594	.221
1 $II r_1 r_1 R_2 T R_2 T$ -----	1	.797	.051	1 $ii r_1 r_1 r_2 t r_2 t$ -----	1	.797	.051
2 $II r_1 r_1 R_2 T r_2 t$ -----	3	1.594	1.240				

$P=0.506$. $\chi^2=25.227$.

Table 10 shows the assumed genotypes for the F_2 plants, together with the observed and expected numbers of each when grouped according to their breeding behavior. The data conform closely to the theoretical, although three of the expected genotypes were not obtained. However, with 51 individuals and 27 different classes, the distribution is considered a very good fit. The values of χ^2 and P are 25.227 and 0.506, respectively.

In order to submit the data to a more critical test, 56 F_3 plants from F_2 plant No. 12 (Table 9) which segregated like the F_1 were tested in the greenhouse during the winter. Data on the breeding

behavior of this family of plants should be equivalent to the data on the breeding behavior of the F_2 plants. Adding these 56 F_3 plants to the 51 F_2 plants tested makes a total of 107 plants which were submitted to the progeny test. With the 107 plants all the assumed genotypes were obtained, though from the 56 F_3 plants 3 expected genotypes were not produced. These 3, however, were different from the 3 not obtained in the F_2 generation. The value of χ^2 for the 107 plants was 27.305 and for P , 0.394. The P value here was no larger than for the F_2 distribution. The probable errors and P values were determined for the total of the F_3 plants in each class of the 27 genotypes which segregated. By referring to Table 9 it will be noted that for all plants heterozygous for one factor the deviations from a 3:1 ratio when compared with their probable errors are in no case too large to be considered as due to other influences than random sampling. Also, for all plants heterozygous for two factors the P values although not high indicate good fits for a 9:3:3:1 ratio. On the other hand the P value (0.026) for plants segregating for three factors is low, indicating a poor fit. Just why the P value here is so low as compared with the P value for the same distribution in the F_2 can not be explained satisfactorily. It will be observed, however, that the yellow-brown class is the one that deviates most. Turning to Table 8 for the F_2 distribution, it may be noted that the number of plants in this class, although a little high, is not excessive. In looking over data presented by Owen (6) on similar crosses the number of plants in this class does not seem to be excessive. It is thought, therefore, that the deviation here is due to chance.

CROSSES INVOLVING EYEBROW-COLOR PATTERN

The inheritance of eyebrow-color pattern has been reported by Nagai and Saito (2) and by Owen (4, 5). Nagai and Saito reported this color pattern to be due to a restriction factor, K , which inhibits or restricts the black color from developing uniformly over the seed coat. Owen changed the nomenclature slightly. The restriction or inhibiting factor was retained but put into his multiple allelomorphs, I , series and called I^k . This I^k factor was reported to be dominant to the recessive i factor which gives self-black or brown colors, but recessive to I^h and I^l , factors for yellow.

The writer has been able to obtain two different crosses which involve the eyebrow-pattern factor. In cross No. 83 (Ogemaw \times Black Eyebrow) the Ogemaw is a self-colored reddish-brown variety. The Black Eyebrow has a brown ground color with a saddle of black over the sides. The F_1 plants bore seeds like the Black Eyebrow parent. In the F_2 four different color types were observed—the black eyebrow, brown eyebrow, self-black, and self-brown. With these four different color types it is evident that at least two factors are involved. A total of 74 F_2 plants was obtained. For a dihybrid ratio with 74 individuals the numbers expected are 41.6:13.9:13.9:4.6. The observed ratio was 39:11:20:4. The χ^2 value for this distribution is 3.55 and the P value 0.32.

In the second cross, No. 183 (Ito San \times Black Eyebrow), involving the eyebrow-pattern color, two F_1 plants were obtained which were given the numbers 183-1 and 183-2. The Ito San parent had a yellow seed coat, a light, brown hilum, and was mottled to a considerable

extent. The Black Eyebrow parent has already been described above. The F_1 plants from these crosses were yellow mottled with black. In the F_2 generation four color types were observed—yellow mottled with black, yellow mottled with brown, black eyebrow pattern, and brown eyebrow pattern. It is evident that at least two factors are involved in this cross and that one of the two is the same as in cross No. 83 and the other is different. Considering the F_2 data from the two F_1 plants (183-1 and 183-2) together there were 150 F_2 plants obtained. For this number of plants, with two pairs of factors involved, the expected ratio is 84.4:28.1:28.1:9.4. The actual ratio was 82:28:25:15. With the exception of the last class represented by the brown eyebrow pattern type, which is a little high, the numbers conform well to the expected. The values for χ^2 and P in this cross are 3.795 and 0.288, respectively. The F_2 data for these crosses are presented in Table 11.

TABLE 11.— F_2 Progenies from crosses between varieties of soybeans which involved the eyebrow-pattern factor

Cross	Mottled		Eyebrow pattern		Self-color	
	Yellow black	Yellow brown	Black	Brown	Black	Brown
Ogemaw \times Black Eyebrow:						
No. 83-1			23	6	8	2
No. 83-2			16	5	12	2
Total observed			39	11	20	4
Expected ^a			41.6	13.9	13.9	4.6
Ito San \times Black Eyebrow:						
No 183-1	43	10	11	9		
No 183-2	39	18	14	6		
Total observed	82	28	25	15		
Expected ^b	84.4	28.1	28.1	9.4		

^a $\chi^2=3.6$. $P=0.3$.

^b $\chi^2=3.8$. $P=0.3$.

The factor I^k suggested by Owen (5) is used to designate the restriction gene in the eyebrow-pattern color, and since the Black Eyebrow variety contains black pigment it must also contain R_1 and R_2 . The Ogemaw variety is self-colored and must contain i . It has already been shown that it contains a factor r_1^o which is recessive to r_1 , and it will be shown later that this factor r_1^o is also recessive to R_1 . Assuming then that the Black Eyebrow variety is of the composition $I^k R_1$ and the Ogemaw variety ir_1^o , the data presented for cross No. 83 can be explained.

In cross No. 183 it has already been shown that the brown hilum of the Ito San variety is due to a pigment factor r_1 , which is recessive to R_1 . Assuming that I restricts the brown pigment to the region of the hilum, the composition of the Ito San variety becomes Ir_1 . By crossing this variety with the variety Black Eyebrow, the phenotypes shown in Table 11 for this cross would be expected in approximately a 9:3:3:1 ratio, which fits the facts observed.

In order to test these crosses further, F_3 plants were grown from 70 of the F_2 plants in cross No. 83 and from 148 F_2 plants in cross No. 183. The complete data for the F_3 generation in these crosses are given in Tables 14 and 15. Tables 12 and 13 show the assumed genotypes for the F_2 plants, together with the observed and expected numbers of each when grouped according to their breeding behavior.

TABLE 12.—*Genotypic distribution of F₂ plants as shown by their breeding behavior in the F₃ generation*[Cross No. 83, Ogemaw (*ii r₁^o R₂TR₂T*) × Black Eyebrow (*I^kI^k R₁R₁ R₂TR₂T*)]

Genotype F ₂ plant	Observed	Expected	$\frac{(o-c)^2}{c}$	Genotype F ₂ plant	Observed	Expected	$\frac{(o-c)^2}{c}$
1 <i>R₁R₁ I^kI^k</i> -----	4	4.38	0.032	2 <i>R₁r₁ ii</i> -----	17	8.76	7.751
2 <i>R₁R₁ I^ki</i> -----	6	8.76	.870	1 <i>r₁r₁ I^kI^k</i> -----	4	4.38	.032
2 <i>R₁r₁ I^kI^k</i> -----	9	8.76	.007	2 <i>r₁r₁ I^ki</i> -----	7	8.76	.354
4 <i>R₁r₁ I^ki</i> -----	17	17.52	.015	1 <i>r₁r₁ ii</i> -----	3	4.38	.634
1 <i>R₁R₁ ii</i> -----	3	4.38	.434				

 $P=0.271$. $\chi^2=9.929$.TABLE 13.—*Genotypic distribution of F₂ plants as shown by their breeding behavior in the F₃ generation*[Cross 183, Ito San (*II r₁r₁ R₂TR₂T*) × Black Eyebrow (*I^kI^k R₁R₁ R₂TR₂T*)]

Genotype F ₂ plant	Observed	Expected	$\frac{(o-c)^2}{c}$	Genotype F ₂ plant	Observed	Expected	$\frac{(o-c)^2}{c}$
1 <i>II R₁R₁</i> -----	4	9.25	2.979	2 <i>II^k r₁r₁</i> -----	18	18.50	0.014
2 <i>II R₁r₁</i> -----	18	18.50	.014	1 <i>I^kI^k R₁R₁</i> -----	12	9.25	.817
2 <i>I^kI^k R₁R₁</i> -----	20	18.50	.122	2 <i>I^kI^k R₁r₁</i> -----	13	18.50	1.635
4 <i>II^k R₁r₁</i> -----	39	37.00	.108	1 <i>I^kI^k r₁r₁</i> -----	15	9.25	3.574
1 <i>II r₁r₁</i> -----	9	9.25	.006				

 $P=0.314$. $\chi^2=9.269$.

In Table 12, for cross No. 83, it may be seen that the class heterozygous for self-black contains an excess of the F₂ genotypes. When these data are compared with data in Table 11 it is noticed that the number of F₂ plants with black is also larger than expected. Since all but two plants in this group were tested, an excess of the segregating self-black plants would be expected. In Table 13, for cross No. 183, it may be observed that the genotypic ratio for the F₂ plants approaches very closely to the expected. The values of χ^2 and P for the F₂ genotypic distribution are 9.93 and 0.27 for cross No. 83 and 9.27 and 0.31 for cross No. 183.

TABLE 14.—*Assumed genotypes together with breeding behavior of F₂ plants tested in the F₃ generation*[Cross No. 83 Ogemaw (*ii r₁^or₁^o R₂R₂*) × Black Eyebrow (*I^kI^k R₁R₁ R₂R₂*)]

Genotype and F ₂ plant No.	Phenotypes observed in the F ₃ generation				Deviation P. E.
	Eyebrow pattern		Self-colored		
	Black	Brown	Black	Brown	
<i>I^kI^k R₁R₁ R₂R₂:</i>					
1-30	12				
1-34	36				
2-2	83				
2-12	44				
<i>I^kI^k r₁^or₁^o R₂R₂:</i>					
1-7		37			
1-20		60			
1-26		84			
2-26		44			
<i>ii R₁R₁ R₂R₂:</i>					
1-11			27		
1-23			56		
2-5			11		
<i>ii r₁^or₁^o R₂R₂:</i>					
1-5				80	
2-7				61	
2-21				43	

TABLE 14.—Assumed genotypes together with breeding behavior of F_2 plants tested in the F_3 generation—Continued[Cross No. 83 Ogemaw ($i i r_1 r_1 R_2 R_2$) \times Black Eyebrow ($I^k I^k R_1 R_1 R_2 R_2$)]

Genotype and F ₂ plant No.	Phenotypes observed in the F ₃ generation				Deviation P. E.
	Eyebrow pattern		Self-colored		
	Black	Brown	Black	Brown	
<i>I^kI^k R₁r₁° R₂R₂:</i>					
1-1	34	11			
1-8	14	8			
1-9	19	12			
1-17	46	19			
1-24	30	16			
1-29	43	14			
1-32	7	1			
1-35	7	7			
2-34	26	7			
Total	226	95			2.82
<i>I^ki R₁R₁ R₂R₂:</i>					
1-21	31		10		
1-36	13		5		
2-24	37		10		
2-32	4		4		
2-35	52		14		
2-31	22		4		
Total	159		47		1.07
<i>I^ki r₁r₁° R₂R₂:</i>					
1-10		15		7	
1-18		6		1	
1-33		33		11	
2-6		16		4	
2-8		96		44	
2-23		19		7	
2-28		49		15	
Total		234		89	1.57
<i>R₁r₁° R₂R₂:</i>					
1-12			24	4	
1-13			16	7	
1-14			38	11	
1-25			80	32	
1-27			61	22	
1-37			28	20	
2-3			53	20	
2-9			14	2	
2-11			1	2	
2-13			30	10	
2-14			36	17	
2-15			31	12	
2-16			12	3	
2-19			48	13	
2-20			51	22	
2-22			50	17	
2-36			33	12	
Total			606	226	2.14
<i>I^ki R₁r₁° R₂R₂:</i>					
1-2	10	2	3	1	
1-3	1	1	2	1	
1-4	21	3	6	2	
1-6	13	3	5	2	
1-15	14	5	4	3	
1-19	21	10	8	7	
1-31	23	9	9	4	
1-38	27	6	8	3	
1-40	42	13	17	2	
2-4	40	16	11	5	
2-10	28	12	9	4	
2-18	38	16	12	5	
2-25	13	4	4	1	
2-27	9	5	2	1	
2-30	31	8	14	4	
2-33	36	8	11	2	
2-37	25	4	14	4	
Total	395	125	139	51	

 $\chi^2=1.79$. P=0.62.

TABLE 15.—Assumed genotypes, together with breeding behavior of F_2 plants tested in the F_3 generation[Cross No. 183 Ito San ($II\ r_1r_1\ R_2R_2$) \times Black Eyebrow ($I^kI^k\ R_1R_1\ R_2R_2$)]

Genotypes and F ₂ plant No.	Phenotypes observed in the F ₃ generation				Deviation P. E.
	Mottled		Eyebrow pattern		
	Yellow black	Yellow brown	Black	Brown	
II R ₁ R ₁ R ₂ R ₂ :					
1-45.....	57				
2-10.....	138				
2-29.....	63				
2-58.....	58				
II r ₁ r ₁ R ₂ R ₂ :					
1-28.....		89			
1-46.....		28			
1-68.....		63			
2-17.....		70			
2-23.....		64			
2-27.....		71			
2-30.....		63			
2-43.....		68			
2-72.....		66			
I ^k I ^k R ₁ R ₁ R ₂ R ₂ :					
1-3.....			61		
1-12.....			48		
1-31.....			104		
1-40.....			49		
1-71.....			64		
1-73.....			65		
2-25.....			61		
2-28.....			68		
2-42.....			58		
2-44.....			65		
2-49.....			49		
2-75.....			59		
I ^k I ^k r ₁ r ₁ R ₂ R ₂ :					
1-2.....				91	
1-10.....				66	
1-18.....				11	
1-33.....				76	
1-35.....				35	
1-38.....				55	
1-47.....				63	
1-59.....				80	
1-63.....				66	
2-9.....				55	
2-13.....				54	
2-19.....				56	
2-21.....				31	
2-24.....				27	
2-65.....				29	
II R ₁ r ₁ R ₂ R ₂ :					
1-1.....	69	25			
1-6.....	42	10			
1-8.....	73	21			
1-20.....	61	26			
1-22.....	96	32			
1-29.....	42	13			
1-41.....	41	13			
1-52.....	45	13			
1-53.....	32	13			
2-15.....	45	12			
2-26.....	45	15			
2-39.....	76	30			
2-41.....	48	10			
2-47.....	111	41			
2-51.....	117	37			
2-63.....	69	21			
2-67.....	74	24			
2-73.....	61	15			
Total.....	1, 147	371			0. 75

TABLE 15.—Assumed genotypes, together with breeding behavior of F_2 plants tested in the F_3 generation—Continued[Cross No. 183 1 to San ($II\ r_1r_1\ R_2R_2$) \times Black Eyebrow ($I^kI^k\ R_1R_1\ R_2R_2$)]

Genotypes and F ₂ plant No.	Phenotypes observed in the F ₂ generation				Deviation P. E.
	Mottled		Eyebrow pattern		
	Yellow black	Yellow brown	Black	Brown	
II ^k R ₁ R ₁ R ₂ R ₂ :					
1-11	59		20		
1-15	59		30		
1-17	103		38		
1-19	80		37		
1-27	77		20		
1-30	37		16		
1-32	77		37		
1-51	78		23		
1-54	81		26		
1-61	106		31		
1-66	72		31		
1-69	48		10		
2-4	89		28		
2-5	46		12		
2-8	105		30		
2-45	22		10		
2-48	44		18		
2-50	72		27		
2-69	83		21		
2-74	29		14		
Total	1,367		479		1.39
II ^k r ₁ r ₁ R ₂ R ₂ :					
1-5		113		33	
1-44		74		28	
1-49		119		35	
1-55		74		16	
1-60		82		22	
1-64		97		33	
1-72		99		35	
2-2		119		35	
2-6		53		17	
2-16		22		9	
2-18		76		32	
2-20		99		45	
2-32		55		14	
2-37		92		28	
2-52		67		13	
2-55		25		4	
2-59		111		37	
2-60		111		29	
Total		1,488		405	1.80
I ^k I ^k R ₁ r ₁ R ₂ R ₂ :					
1-21			64	18	
1-23			91	38	
1-25			26	9	
1-50			50	17	
1-58			81	18	
2-22			95	32	
2-33			75	26	
2-46			19	7	
2-61			64	23	
2-62			99	44	
2-64			36	13	
2-76			104	22	
2-77			45	10	
Total			849	277	.46
II ^k R ₁ r ₁ R ₂ R ₂ :					
1-4	48	16	19	6	
1-7	33	11	11	4	
1-9	13	7	4	2	
1-13	57	15	16	8	
1-14	87	29	20	12	
1-16	57	26	18	6	
1-24	43	10	14	4	

TABLE 15.—Assumed genotypes, together with breeding behavior of F_2 plants tested in the F_3 generation—Continued[Cross No. 183 I to San ($II\ r_1r_1\ R_2R_2$) \times Black Eyebrow ($I^kI^k\ R_1R_1\ R_2R_2$)]

Genotypes and F ₂ plant No.	Phenotypes observed in the F ₃ generation				Deviation P. E.
	Mottled		Eyebrow pattern		
	Yellow black	Yellow brown	Black	Brown	
<i>II^k R₁R₁ R₂R₂—Continued.</i>					
1-26.....	51	20	16	7	-----
1-34.....	25	11	9	7	-----
1-36.....	22	9	19	4	-----
1-37.....	47	10	10	6	-----
1-39.....	25	6	12	3	-----
1-42.....	67	20	20	6	-----
1-43.....	79	17	15	7	-----
1-48.....	38	28	18	4	-----
1-56.....	70	16	22	9	-----
1-57.....	36	12	12	3	-----
1-62.....	77	15	24	12	-----
1-65.....	97	17	28	9	-----
1-67.....	34	7	8	2	-----
1-70.....	62	22	15	11	-----
2-1.....	19	7	7	0	-----
2-3.....	99	40	40	15	-----
2-7.....	58	17	16	3	-----
2-11.....	41	19	12	4	-----
2-14.....	43	13	15	5	-----
2-31.....	33	22	12	1	-----
2-34.....	35	13	10	3	-----
2-35.....	43	9	13	3	-----
2-38.....	94	22	14	9	-----
2-40.....	111	24	24	10	-----
2-53.....	34	8	9	1	-----
2-54.....	91	22	30	9	-----
2-56.....	58	19	17	10	-----
2-57.....	94	32	20	12	-----
2-58.....	49	11	21	7	-----
2-66.....	45	15	13	6	-----
2-68.....	55	16	15	11	-----
2-71.....	71	23	23	8	-----
Total.....	2, 144	656	641	249	-----

 $\chi^2=9.3$. $P=0.03$.

By referring to Table 14 for cross No. 83 and Table 15 for cross No. 183, it may be seen that the deviation from a 3:1 ratio for each of the classes segregating for one pair of factors when compared with its probable error is not too large for a good fit. Tables 14 and 15 also contain data for F_2 plants of the above crosses segregating as the F_1 . The values of χ^2 and P for cross No. 83 (Table 14) are 1.79 and 0.62, respectively, and for cross No. 183 (Table 15) are 9.32 and 0.03, respectively. In the last case the fit is poor, but since the rest of the F_3 data and the results obtained for the F_2 generation conform well to the expected, it is thought that the inheritance can well be explained by assuming two independent factors.

SELECTION INVOLVING YELLOW AND BROWN SEED-COAT COLORS

Owen (5) has reported results of crosses between varieties of soybeans with yellow seed coats and varieties with brown seed coats. In his work approximately three plants bearing yellow seeds to one with self-colored seeds were obtained. Two restriction factors for pigment I^h or I^t and their recessive allelomorph were assumed in order to explain his findings. When a variety having a yellow seed coat and

black hilum was crossed with a brown-seeded variety, F_2 plants bearing self-colored black seeds and plants bearing self-colored brown seeds were obtained. When a variety having a yellow seed coat and a brown hilum was crossed with a variety having a brown seed coat only F_2 plants with yellow seeds and brown hilums or self-brown seeds were obtained.

During these investigations a probable natural hybrid, selection No. 31, was found to be segregating for yellow and brown seed-coat color. The original plant, selection No. 31, bore yellow mottled brown seeds and had tawny pubescence. From the seeds of this plant 43 progenies (the F_2 plants) were grown. Of these, 28 bore yellow seeds which were mottled with brown and 15 seeds which were self-brown. All the F_2 plants had tawny pubescence. For a 3:1 ratio one would expect from 43 plants, 32.25 to bear yellow seeds mottled with brown and 10.75 to bear self-brown seeds. The deviation 4.25 ± 1.92 is considered a fairly close fit, and the original plant is thought to have been hybrid for a single pair of factors. Using I as the restriction factor for pigment color, and its recessive allelomorph i to designate self-color, the plant must have had the genetic constitution Ii . A natural cross between the Ito San variety and the Soysota variety would give such a genotype.

ALLELOMORPHIC SERIES OF FACTORS WHICH RESTRICT BLACK AND BROWN PIGMENTS

Owen (5) has reported a series of allelomorphs I^h , I^i , I^k , and i which have certain inhibiting effects upon the development of pigment colors in the seed coat. Data taken from certain crosses obtained in the present studies show rather conclusively that such a series does exist. However, the writer has not been able to differentiate between I^h and I^i . Owen also had some trouble in making this classification, especially where mottling took place to any extent. All progenies from crosses used in the present studies where yellow color was involved mottled badly. Possibly this is the reason that I^h and I^i could not be distinguished. Since the author has not been able to differentiate between I^h and I^i , any plants with yellow seed coat regardless of the color of the mottling or color of hilum have been designated as I . The series used in this paper is I , I^k , and i .

The evidence for such a series is obtained from F_2 data on the following crosses:

Ito San (Ii) \times Black Eyebrow (I^kI^k)—3 plants with yellow-mottled seeds to 1 with the eyebrow pattern.

Black Eyebrow (I^kI^k) \times Ogemaw (ii)—3 plants with the eyebrow pattern to 1 with self-colored seeds.

Manchu (Ii) \times Wisconsin Black (ii)—3 plants with yellow-mottled seeds to 1 with self-black.

The breeding behavior observed in the above crosses is good evidence that the three factors I , I^k , and i are allelomorphic. No other explanation would likely give only the two types which go into the cross in the F_2 generation. Data for the first two combinations are shown in Table 11 and for the last combination in Table 8. These crosses lend support to Owen's hypothesis that a multiple allelomorphic series exists at the I locus by bringing in data from one combination, Black Eyebrow (I^kI^k) \times Ogemaw (ii), which Owen did not obtain.

ALLELOMORPHIC SERIES OF FACTORS WHICH INFLUENCE THE INTENSITY OF BLACK AND BROWN PIGMENTS

Owen (5) has postulated that an allelomorphous series which influences the pigment color is situated near the t locus for pubescence color. In these studies no evidence was obtained which indicated an allelomorphous series at this locus. There is, however, considerable indication that such a series exists at the R_1 locus. Evidence for such a series was obtained from F_2 data from the following crosses:

Manchu (R_1R_1) \times Ito San (r_1r_1)—3 plants bearing seeds with black pigment to 1 with light brown.

Manchu (R_1R_1) \times Wisconsin Black (R_1R_1)—all plants bearing seeds with black pigment.

Wisconsin Black (R_1R_1) \times Soysota (r_1r_1)—3 plants bearing seeds with black pigment to 1 with light brown.

Soysota (r_1r_1) \times Ogemaw ($r_1^o r_1^o$)—3 plants bearing seeds with light-brown pigment to 1 with reddish brown.

Black Eyebrow (R_1R_1) \times Ogemaw ($r_1^o r_1^o$)—3 plants bearing seeds with black pigment to 1 with reddish brown.

Black Eyebrow (R_1R_1) \times Ito San (r_1r_1)—3 plants bearing seeds with black pigment to 1 with light brown.

Data for the first combination may be obtained by referring to Table 1; for the second combination, Table 8; for the third, paragraph involving black and brown seed coat, on page 835; for the fourth, Table 2; and for the fifth and sixth, Table 11.

With the above combinations behaving as they do it seems advisable to assume that an allelomorphous series exists at the R_1 locus. No other explanation would likely give only the types which go into the crosses in the F_2 generation. Two questions which might arise are: (1) Is Black Eyebrow really of the constitution R_1R_1 , and, (2), what would happen should Wisconsin Black be crossed with Black Eyebrow? In the discussion of cross No. 83, it was shown that four different color types appear in the F_2 generation—the black eyebrow, reddish-brown eyebrow, self-black, and self-reddish brown. Without R_1 in the Black Eyebrow variety no self-blacks could come out of this cross because it is crossed with a variety Ogemaw which contains no black pigment. No cross has been made between Wisconsin Black and Black Eyebrow, but from the same cross, No. 83, 6 plants of the Black Eyebrow type segregated for approximately 3 plants of the Black Eyebrow type to 1 self-black. From this cross 17 self-black F_2 plants of the genetic constitution $R_1r_1^o$ were obtained which gave a good 3:1 ratio of self-blacks and reddish browns. The data for these combinations may be seen by referring to Table 14.

DISCUSSION

As previously stated, a number of workers, Nagai (1), Nagai and Saito (2), Woodworth (7), and Owen (4, 5) have studied these pigment colors genetically. Certain similarities have been observed in their findings which may be mentioned.

Nagai (1) reported studies of seed-coat inheritance from crosses involving the pigments discussed in this paper. According to his hypothesis two complementary factors, C and R , were responsible for the formation of black pigment. With CR the color was black, with cR the color was imperfect black, with Cr the color was brown, and with both recessives, cr , the color was buff.

Nagai and Saito (2) reported three factors, H , I , and K , which inhibit the black or brown pigment. With H present no black or brown pigment develops, and the seed coat is yellow or green. The factor I inhibits or restricts the pigment to the hilum, and K restricts the pigment to give the eyebrow pattern.

Woodworth (7) in a study of black and brown pigments in the hilum observed two complementary factors for the inheritance of black and brown pigments. These two color factors were designated H and B .

Owen (4, 5) in an extensive study of soybean inheritance observed two factors, R_1 and R_2 , for black pigments. He also reported an allelomorph series of factors which restrict the pigments entirely or to certain areas on the seed coat.

It can not be definitely determined whether the factors reported by the various workers are the same. There are, however, many resemblances between certain of the factors which will be brought out later in the discussion.

As stated earlier, the work of Owen is more extensive than that of previous investigators. Therefore to avoid confusion in nomenclature it seems preferable to use his symbols as far as possible in explaining data collected in these studies. Before advancing further in the discussion the assumed genotypes used for all of the different crosses should be presented. These are as follows:

Wisconsin Black ($ii R_1 R_1 R_2 TR_2 T$) \times Mandarin ($II r_1 r_1 r_2 tr_2 t$).
 Ogemaw ($ii r_1^o r_1^o R_2 TR_2 T$) \times Soysota ($ii r_1 r_1 R_2 TR_2 T$).
 Black Eyebrow ($I^k I^k R_1 R_1 R_2 TR_2 T$) \times Ogemaw ($ii r_1^o r_1^o R_2 TR_2 T$).
 Manchu ($II R_1 R_1 R_2 TR_2 T$) \times Ito San ($II r_1 r_1 R_2 TR_2 T$).
 Ito San ($II r_1 r_1 R_2 TR_2 T$) \times Black Eyebrow ($I^k I^k R_1 R_1 R_2 TR_2 T$).
 Manchu ($II R_1 R_1 R_2 TR_2 T$) \times Wisconsin Black ($ii R_1 R_1 R_2 TR_2 T$).
 Manchu ($II R_1 R_1 R_2 TR_2 T$) \times Wilson ($ii R_1 R_1 R_2 TR_2 T$).
 Wisconsin Black ($ii R_1 R_1 R_2 TR_2 T$) \times Soysota ($ii r_1 r_1 R_2 TR_2 T$).
 Natural hybrid No. 10 ($II r_1 r_1 R_2 TR_2 T$) \times ($II r_1 r_1 r_2 tr_2 t$).⁵
 Natural hybrid No. 31 ($ii r_1 r_1 R_2 TR_2 T$) \times ($II r_1 r_1 R_2 TR_2 T$).⁵

In the above hypothesis it is assumed that there is an allelomorph series at the I locus. Gene I is a restriction factor which must be present in all varieties with yellow seed coats. I^k is an allelomorph to I which restricts black or brown color so as to give the eyebrow-color pattern. Gene i is a recessive allelomorph to both I and I^k and must be present in order to get self-colors of any kind. A second series of allelomorphs (R_1 , r_1 , and r_1^o) is assumed at the R_1 locus. The factor R_1 causes the development of anthocyanin pigment. It is also complementary to R_2 , giving the intense black color. With $R_1 r_2$ the color is imperfect black. Gene r_1 is recessive to R_1 , and in the presence of R_2 gives a light brown color. With $r_1 r_2$ the color is buff. The factor r_1^o is recessive to either R_1 or r_1 . When present with R_2 it gives a rich reddish-brown pigment. No crosses involving r_2 and r_1^o have been obtained. R_2 is closely lined with T , a factor for tawny pubescence, and no crossing over has been observed.

Although the symbols used by Owen (5) have been followed as closely as possible it has been necessary to make some departures in order to explain satisfactorily data gathered during this study. The first change which will be discussed is the difference in the I series of

⁵ The last two combinations were natural hybrids and the parents are unknown. The genetic composition has been assumed from their breeding behavior.

allelomorphs. In his hypothesis Owen has used two factors in this series I^h and I^i which the writer has been unable to use. Quoting from Owen (5, p. 56):

I^h under the proper environmental conditions inhibits all pigment formation in the seed coat; I^i inhibits pigments to the hilum. * * * It should be clearly kept in mind that environmental conditions unfavorable for pigment formation are necessary in order for these restriction factors to fully express themselves.

Nagai and Saito (2) have also described a factor H which acts very much like the I^h factor described by Owen.

As already shown, the present writer has been unable to make such a classification. Almost all plants with yellow seed coats used in these studies have been mottled to a considerable extent. Occasionally plants occurred which were reasonably free from mottling. Others appeared which were extremely mottled, but when tested in the F_2 there was no indication of these differences being genetic. It has also been noticed that there was just about as much variation in mottling among seeds on a plant as there was among the different plants. During the course of this study there has been no occasion for distinguishing between I^h and I^i . Therefore, no attempt has been made to separate the two factors. I alone has been used to designate the restriction of black and brown pigments giving the yellow or yellow-mottled color. It is quite possible that plants used for these crosses were genetically different from those used by Owen. Another possibility is that environmental conditions at Ames, Iowa, were such that the color pigments were brought out much more distinctly than they were at Madison, Wis. It has been shown by Hollowell⁶ and Owen (3) that different environmental conditions do play an important part in the degree of mottling. This may account for different degrees of mottling which the author has often noticed on the same plant and which has been reported by Woodworth and Cole (9). Hollowell,⁷ and Owen (3). Another point which might be mentioned is that all plants used in these studies were spaced 4 to 6 inches apart in the rows and grown on land which was fairly fertile. Both spacing and soil fertility have been found by Owen and Hollowell to influence the degree of mottling.

Some changes have been made in the uses of the two R factors for black and brown pigments. In these studies the author has found it necessary to postulate a series of allelomorphs at the locus of R_1 , but it has not been necessary to use the allelomorphic series at the locus of R_2 , as postulated by Owen (5). The factor R_1 —which is probably the R factor reported by Nagai (1), the B factor reported by Woodworth (7), and the R_1 factor reported by Owen (5)—causes the development of anthocyanin pigment. However, it does not, when present alone, give the intense-black color which we are accustomed to see in black varieties of soybeans. Only when R_1 appears in the zygote with R_2 is the intense black color produced. In combination with r_2 , R_1 causes anthocyanin pigment to develop, but it gives a dilute black instead of the ordinary black color. The factor R_2 is probably the same as the chromongen factor C described by Nagai (1), the H factor described by Woodworth (7), and the R_2 factor described by Owen (5). The factor R_2 , although described by Owen as being a

⁶ HOLLOWELL, E. A. FACTORS INFLUENCING THE MOTTLING OF THE SOYBEAN SEED COAT. 1924. Unpublished master's thesis. Copy on file, library, Iowa State College, Ames.

⁷ HOLLOWELL, E. A. Op. cit.

factor for black pigment, does not cause the development of anthocyanin pigment alone, but acts in a complementary way with R_1 to produce black. This fact has also been observed by Nagai and Saito (2) and Woodworth (7). When R_2 is present in the zygote with r_1 , a light-brown color is produced. With both r_1 and r_2 the color is buff.

As R_1 and R_2 are used in this paper, data from all crosses involving black and brown pigment colors in the seed coat thus far reported can be explained. However, in one cross, No. 142 (Selection No. 3 \times Selection No. C 3) reported by Owen, a slight modification must be made. In this cross flower color is also involved, and in order to explain the data presented by Owen with the hypothesis here presented it is necessary to assume that W , the factor for purple-flower color, is also complementary to R_1 to give the imperfect-black color in the seed coat. In using the hypothesis presented by Owen the W factor is considered as complementary to r_2 instead of R_1 . This assumption brings in certain complications which are not easily explained. Since the main difference in the use of R_1 and R_2 centers around this one cross, it is thought advisable to discuss the cross from the standpoint of the two hypotheses. According to Owen's hypothesis, Selection No. 3 possessed the following constitution: $ii\ r_1r_1\ R_2TR_2T\ WW$ and Selection No. C 3, $I^hI^h\ r_1r_1\ r_2tr_2t\ ww$. For this combination the F_2 phenotypes would be as follows:

27	$I^h\ r_1\ R_2TW$ -----	Yellow, mottled with black—tawny pubescence—purple flower.
9	$I^h\ r_1\ R_2Tww$ -----	Yellow, mottled with black—tawny pubescence—white flower.
9	$I^h\ r_1\ r_2tW$ -----	Yellow, mottled with imperfect black—Gray pubescence—purple flower.
3	$I^h\ r_1\ r_2tww$ -----	Yellow, mottled with buff—gray pubescence—white flower.
9	$i\ r_1\ R_2TW$ -----	Self-black—tawny pubescence—purple flower.
3	$i\ r_1\ R_2Tww$ -----	Self-black—tawny pubescence—white flower.
3	$i\ r_1\ r_2tW$ -----	Self-imperfect black—gray pubescence—purple flower.
1	$i\ r_1\ r_2tww$ -----	Self-buff—gray pubescence—white flower.

The data presented by Owen are explained very satisfactorily by the above hypothesis, but when one considers the genetic relationship of the two varieties used in this cross along with other crosses which are similar, certain conditions arise which can not well be explained.

By observing the phenotypes it will be noticed that $I^h\ r_1\ r_2t\ W$ has been used to designate yellow mottled with imperfect black; but this is the same formula which has to be used for the Mandarin variety. The Mandarin variety has a yellow seed coat. It, then, must contain I^h , the restriction factor.⁸ It has buff pigment in the hilum and is represented by Owen as well as by the present writer as containing r_1 . It has gray pubescence; therefore it must contain r_2 because r_2 is completely linked with t , factor for gray pubescence. Finally, the Mandarin variety has purple flowers and therefore must contain W . Accordingly, the formula $I^h\ r_1\ r_2t\ W$ in the Mandarin variety means yellow mottled with buff. Here it is assumed to mean yellow mottled with imperfect black. A second inconsistency in the hypothesis is found in the phenotype $i\ r_1R_2TW$, which here is used to designate self-black. But this is the same formula which has to be used in the Soy-sota variety with a brown seed coat. Soysota is self-colored and

⁸ According to the hypothesis used by the present author I^h should be I .

therefore it must have i . It has been designated by Owen as well as by the writer as having r_1 , and it must contain R_2TW because it has tawny pubescence and purple flowers. A third inconsistency in Owen's hypothesis is found in the phenotype $i r_1 r_2t W$, which here is used to designate self-colored imperfect black, but in certain crosses reported by Owen this formula has been used to designate plants which bore buff-colored seeds and purple flowers. There is still one other feature of the hypothesis which seems to be rather unsatisfactory. This will be observed in the phenotype $I^h r_1 R_2TW$, which here is used to designate yellow mottled with black. In Ito San, a yellow-seeded variety with a brown hilum which often mottles brown, I^h and r_1 have been reported by Owen to be present. The studies made by the present author bear out this assumption. But the Ito San variety has purple flowers and tawny pubescence. It therefore should have the genetic constitution $I^h r_1 R_2TW$. Owen, too, has noticed this fact, and in order to explain his data when the Ito San variety is crossed with Selection No. 3 (the black parent) of the above cross, he assumed an allelomorphic series to exist at the R_2 locus, giving the Ito San variety the formula $I^h r_1 r_2' TW$.

These rather inconsistent uses of formulae seem to be unnecessary, and the author is presenting a hypothesis for the cross which he believes answers all requirements in this cross and at the same time is in agreement with the factorial composition of other crosses used by previous workers as well as in the present studies.

The genetic constitution of Owen's Selection No. 3 (black parent) is assumed to be $ii R_1R_1 R_2TR_2T WW$ and for Selection No. C 3 (yellow parent), $II R_1R_1 r_2tr_2t ww$. The F_2 phenotypic ratio is as follows:

27	$I R_1 R_2T W$	-----	Yellow, mottled with black—tawny pubescence—purple flower.
9	$I R_1 R_2T w$	-----	Yellow, mottled with black—tawny pubescence—white flower.
9	$I R_1 r_2t W$	-----	Yellow, mottled with imperfect black—gray pubescence—purple flower.
3	$I R_1 r_2t w$	-----	Yellow, mottled with buff—gray pubescence—white flower.
9	$i R_1 R_2T W$	-----	Self-black—tawny pubescence—purple flower.
3	$i R_1 R_2T w$	-----	Self-black—tawny pubescence—white flower.
3	$i R_1 r_2t W$	-----	Self-imperfect black—gray pubescence—purple flower.
1	$i R_1 r_2t w$	-----	Self-buff—gray pubescence—white flower.

With the above hypothesis it is necessary to assume that the factor W for purple flower color is complementary to R_1 , giving the imperfect-black color in the seed coat. With R_1 and w the color of the pigment is buff. It may be said that the imperfect black shows considerable buff pigment and that a slight diluting of the imperfect black by w would produce a buff color. This diluting effect is in accord with other observations. Woodworth (8) has noticed that the purple flower color was closely associated with purple hypocotyl and that with white flowers no purple pigment was developed. This point, however, is not a disagreement since Owen has had to assume in his hypothesis that W was complementary to r_2 to produce the imperfect-black color of the seed coat.

With the hypothesis presented in this paper the results obtained by crossing the plant from the Ito San variety with Selection No. 3 (Owen (5)) can be explained without assuming an allelomorphic series

at the R_2 locus. Also, a particular color can be designated by the same formula regardless of the variety in which it appears.

It may be well to mention that the author has crossed the Ito San variety with the varieties Black Eyebrow and Manchu, both of which contain the factor R_2 . In neither case was there any evidence of a segregation for R_2 , and, therefore, no indication of an allelomorphic series at the R_2 locus.

SUMMARY

Data for the F_2 and F_3 generations are presented to show the inheritance of yellow, black, imperfect-black, light-brown, reddish-brown, and buff colors in the seed coat of soybeans.

Two complementary factors R_1 and R_2 cause the development of black pigment. With R_1r_2 the color is a dilute or imperfect-black, with r_1R_2 the pigment color is light-brown, and with r_1r_2 the color is buff.

A series of allelomorphs, R_1r_1 and r_1° , has been found to exist at the R_1 locus. R_1 with R_2 produces black pigment, r_1 with R_2 light-brown, and r_1° with R_2 a reddish-brown color.

Two pigment restriction factors I and I^* form an allelomorphic series with i . I restricts any of the pigment colors to the hilum or regions around the hilum. I^* restricts pigments in such a way as to give the eyebrow pattern. The factor i has no inhibiting effect and when present in the homozygous condition the seeds are self-colored.

The factor R_2 , if not the same as T , the factor for tawny pubescence, is closely linked with T . No crossing over between the two factors has been observed in these studies.

LITERATURE CITED

- (1) NAGAI, I.
1921. A GENETIC-PHYSIOLOGICAL STUDY ON THE FORMATION OF ANTHOCYANIN AND BROWN PIGMENTS IN PLANTS. Jour. Col. Agr., Imp. Univ. Tokyo 8: 1-92, illus.
- (2) ——— and SAITO, S.
1923. LINKED FACTORS IN SOY-BEAN. Japan. Jour. Bot. 1: 121-136.
- (3) OWEN, F. V.
1927. HEREDITARY AND ENVIRONMENTAL FACTORS THAT PRODUCE MOTTLING IN SOY BEANS. Jour. Agr. Research 34: 559-587, illus.
- (4) ———
1927. INHERITANCE STUDIES IN SOYBEANS. II. GLABROUSNESS, COLOR OF PUBESCENCE, TIME OF MATURITY, AND LINKAGE RELATIONS. Genetics 12: 519-529.
- (5) ———
1928. INHERITANCE STUDIES IN SOYBEANS. III. SEED-COAT COLOR AND SUMMARY OF ALL OTHER MENDELIAN CHARACTERS THUS FAR REPORTED. Genetics 13: 50-79, illus.
- (6) PIPER, C. V., and MORSE, W. J.
1923. THE SOYBEAN. 329 p., illus. New York and London.
- (7) WOODWORTH, C. M.
1921. INHERITANCE OF COTYLEDON, SEED-COAT HILUM, AND PUBESCENCE COLORS IN SOYBEANS. Genetics 6: 487-553, illus.
- (8) ———
1923. INHERITANCE OF GROWTH HABIT, POD COLOR, AND FLOWER COLOR IN SOYBEANS. Jour. Amer. Soc. Agron. 15: 481-495, illus.
- (9) ——— and COLE, L. J.
1924. MOTTLING OF SOYBEANS. Jour. Heredity 15: 349-354, illus.

THE NORMAL LIMITS OF VARIATION OF THE METHYLENE-BLUE REDUCTION TEST¹

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INTRODUCTION

The methylene-blue reduction or reductase test as a means of judging the sanitary quality of milk has met with great popularity in this country. This method of analysis has the advantages of being very simple to operate, easy to interpret, and requires very little expense for materials. Many of the small milk plants not equipped with elaborate facilities necessary to make agar-plate counts are using the methylene-blue reduction test as a means of detecting undesirable sources of milk supply. In many cities it has been adopted as a part of the system of regulatory control of the milk supply. Milk inspectors in the larger cities frequently use this test for general survey work and supplement it with other more elaborate tests.

The wide use of the test under many conditions has led to many modifications of the procedure, which for the most part are designed to render it even more simple and practical. Many of those who use this test are interested only in a rather rough approximation of quality; hence, extreme accuracy in measuring the ingredients for the test and strict aseptic precautions have not been deemed essential. In some dairy plants the 10 c. c. of milk used in the test is measured by means of a dipper instead of a pipette; in others, graduated test tubes are used. Instead of using bacteriologically sterile glassware, as is necessary in the plate count, the test tubes are placed in boiling water a few minutes, and the measuring dippers when not in use are kept either in hot water or in a disinfectant solution. Although these practices are admittedly inaccurate, the errors are commonly regarded as of no practical significance.

The preparation of the methylene-blue solution has been simplified by the use of specially prepared tablets that contain a standard amount of the dye, thus eliminating the necessity for expensive chemical balances to weigh small quantities of the powder. One tablet is dissolved in 200 c. c. of water which has been partially sterilized by boiling for a few minutes.

The present investigation was designed primarily to determine the expected limits of normal variation of the methylene-blue test in order to afford more concrete evidence of its accuracy and reliability. If a sample of milk reduces the methylene blue in two and one-half hours, may a second determination be expected to give the same result? If variations are observed, are they likely to be a few minutes or one or two hours? In addition to answering these questions an effort was made to measure the effect of the modifications of technic that have been adopted as a means of simplifying the test.

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If a proposed modification simplifies the test at the expense of accuracy, it is not to be recommended unless it can be shown that the variation introduced by the simplified technic is insufficient in magnitude to be of practical significance.

REVIEW OF LITERATURE

Thornton and Hastings² found that the methylene-blue reduction was dependent upon the dissolved oxygen and that a concentration of 1:200,000 of methylene blue gave approximately the same results as 1:100,000. They concluded that extreme accuracy in measuring the sample is not necessary if reduction occurs in less than five and one-half hours. They, like Prucha and Ambrose, as reported by Mumford,³ found only a very general correlation between the reductase test and the plate count.

A report from the Wisconsin station⁴ indicates that there is considerable variability in the methylene blue from different sources. The prepared tablets were found to be sufficiently uniform for practical purposes, and the adoption of these was recommended.

Troy⁵ compared the methylene-blue reduction and direct microscopic methods of analysis and reported a correlation of 86.2 per cent ($r = 0.862$).

Results obtained by Martin⁶ at the Pennsylvania Agricultural Experiment Station indicate a close agreement of the methylene-blue reduction test and the sediment test for grading milk. Thus, of the samples placed in the first grade by the sediment test, 82 per cent were similarly classified by the methylene-blue reduction test.

PLAN OF EXPERIMENT

In order to study the normal variation which may be expected with the reductase test, relatively large numbers of determinations were made from the same sample of milk. Similarly, in studying the effect of proposed modifications, a relatively large number of parallel tests were made with the same sample of milk, two or more methods being used. Extreme care was exercised to control all variables except those under observation.

In order to prevent a marked change in the number of microbes in the milk during the time required to set up the experiment, the following procedure was adopted in all cases. The milk to be used in the experiment was first chilled in iced water for 20 to 30 minutes. The chilled milk was agitated while the requisite number of 10 c. c. portions were being placed in the tubes. Only 20 tubes were filled at a time, and the basket of filled tubes was immediately replaced in the iced water. As soon as the required number of tubes were thus prepared, the methylene blue was added to each tube. The basket of tubes was immediately placed in a water bath at 37° C. and the time recorded. By setting up a few tubes at a time and by keeping

² THORNTON, H. R., and HASTINGS, E. G. SOME ASPECTS OF THE METHYLENE-BLUE REDUCTION TEST OF MILK. *Jour.* 13⁷ 62-63. 1927.

³ MUMFORD, H. M. A YEAR'S PROGRESS IN SOLVING SOME FARM PROBLEMS IN ILLINOIS. III. *Agr. Expt. Sta. Ann. Rpt.* 40: 158-159. 1927.

⁴ RUSSELL, H. L., MORRISON, F. B., and EBLING, W. H. NEW PAGES IN FARM PROGRESS, METHYLENE BLUE REDUCTION TEST STANDARDS. *Wis. Agr. Expt. Sta. Ann. Rpt.* 1923-24: 71-72. 1925. (Bul. 373.)

⁵ TROY, H. C. A COMPARISON OF THE METHYLENE-BLUE TEST AND THE DIRECT MICROSCOPIC COUNT IN GRADING MILK AT MILK PLANTS. *Jour. Dairy Sci.* 8: 282-285. 1925.

⁶ MARTIN, W. H. JUDGING THE QUALITY OF MILK BY THE METHYLENE-BLUE REDUCTION TEST, SEDIMENT TEST, AND BACTERIA COUNT (PLATE METHOD). *Penn. Agr. Expt. Sta. Ann. Rpt.* 38: 23. 1925 (Bul. 196.)

the milk in iced water until the methylene blue was added the variation in the reduction time caused by bacterial growth was held to a minimum. In all but three experiments the elapsed time between placing the first and last basket of tubes in the 37° C. water bath was about 30 minutes. In the three exceptions the average reduction time of the milk was so short that it was necessary to complete the reduction test on one basket of tubes before adding the methylene blue to another.

The variation in the reduction time for the replicate tests on the same sample of milk was calculated and expressed in most cases as a coefficient of variability. Other statistical devices were resorted to as occasion seemed to demand.

RESULTS

In Table 1 are recorded the results of 1,774 tests on 19 samples of milk. From 75 to 100 tests were made on each sample; in some cases 20 to 50 tests were made by two or more methods, using the same sample of milk.

TABLE 1.—Variations of the methylene-blue reduction test in the examination of various samples of milk

Experiment No.	Tests	Reduction, time			Coefficient of variability	3.2 times the probable error	Plate count per cubic centimeter	Modification of methods in reductase test
		Extremes		Average				
		Low	High					
	Number	Minutes	Minutes	Minutes	Per cent	Minutes		
1-----	80	25	39	29	8.9	6	400,000	10 c. c. pipette.
2a-----	50	31	44	36	10.5	8	300,000	10 c. c. cup.
2b-----	50	42	55	52	8.9	10	300,000	Graduated test tube.
3-----	75	28	50	40	13.0	11	5,000,000	Do.
4-----	80	79	106	93	8.5	17	270,000	10 c. c. pipette.
5a-----	20	71	74	72	1.4	2	4,000,000	0.5 c. c. methylene blue.
5b-----	20	98	104	102	2.0	4	4,000,000	0.8 c. c. methylene blue.
5c-----	20	113	127	119	4.4	11	4,000,000	1 c. c. methylene blue.
5d-----	20	125	133	129	2.0	6	4,000,000	1.2 c. c. methylene blue.
5e-----	20	132	139	135	1.9	5	4,000,000	1.5 c. c. methylene blue.
6a-----	50	105	115	106	1.6	4	3,000,000	Dye solution prepared from tablet.
6b-----	50	107	112	108	1.8	4	3,000,000	Dye solution prepared from powder.
7-----	80	135	171	151	5.7	19	25,000	10 c. c. pipette.
8-----	86	157	164	161	1.2	4	-----	Do.
9a-----	50	201	223	209	2.3	10	200,000	10 c. c. cup.
9b-----	48	165	242	219	7.0	33	200,000	10 c. c. pipette.
9c-----	50	228	241	232	1.8	9	200,000	Graduated test tube.
10a-----	52	211	254	241	3.3	7	-----	Glassware in boiling water 15 minutes.
10b-----	51	245	269	257	1.8	4	-----	Glassware in 20 pounds steam 30 minutes.
11-----	100	233	286	252	10.9	23	-----	10 c. c. cup.
12-----	80	371	408	383	3.4	28	30,000	10 c. c. pipette.
13-----	60	356	407	390	2.6	22	11,000	Do.
14a-----	20	364	386	374	1.6	13	19,000	0.5 c. c. methylene blue.
14b-----	20	390	404	397	1.1	9	19,000	0.8 c. c. methylene blue.
14c-----	20	399	421	408	1.5	13	19,000	1 c. c. methylene blue.
14d-----	20	408	428	419	1.8	10	19,000	1.2 c. c. methylene blue.
14e-----	20	413	439	426	1.6	15	19,000	1.5 c. c. methylene blue.
15-----	82	381	440	419	3.1	28	37,000	10 c. c. pipette.
16a-----	50	384	443	420	4.9	44	14,000	Dye solution prepared from powder.
16b-----	50	393	474	433	5.0	46	14,000	Dye solution prepared from tablet.
17-----	100	404	508	462	4.3	43	60,000	10 c. c. cup.
18-----	100	409	561	484	6.8	71	8,000	Do.
19-----	100	319	550	490	6.9	72	28,000	Graduated test tube.

The data include the extremes, the average reduction time, the coefficient of variability, the probable error multiplied by the factor 3.2, and the plate count for each sample of milk. Any modifications of the usual procedure for the test are designated in the last column on the right. Where two or more modifications were applied to

single sample of milk, the subdivisions of the experiment are designated by a number and a letter (i. e., 2a, 2b, etc.).

Since the effect of the various modifications is brought out in a subsequent discussion, Table 1 is intended to show only the general range and degree of variability of the methylene-blue reduction test and its relation to the plate count. The experiments are arranged approximately in the order of their average reduction time. The probable error multiplied by the factor 3.2, as given in column 7, establishes the limits within which there is a 30 to 1 chance that a subsequent determination will fall. The results with sample No. 1, for example, indicate a practical certainty (30 to 1 chance) that the reduction time of a single test on this milk will not be in error more than six minutes.

A general survey of the figures in the column showing the coefficient of variability reveals that in 14 cases this value was 2 per cent or less. In general these results indicate a very satisfactory degree of uniformity.

With a few exceptions the samples requiring from one and one-half to seven hours for reduction (5e-14) show relatively lower degrees of variation than those requiring more or less time for reduction. In other words, when the reduction time is very short or very long, greater variation in results may be expected.

Some idea of the reliability of the reduction test may be secured by a study of the extremes of variation. Thus, there are only two instances (9b and 11) among the samples requiring less than 7 hours for reduction in which the greatest variation was more than 30 minutes from the average reduction time. In other words, with but two exceptions, if a single test had been accepted on any of the first 14 samples of milk, the result would not have been more than 30 minutes in error. In 6 of the 14 samples requiring less than 7 hours for reduction, the widest deviation from the average was 10 minutes. In the samples requiring from 7 to 8 hours for reduction the deviation from the average ranged from 40 to 95 minutes.

A more concrete idea of the reliability of this test may be obtained from the column showing the values for 3.2 times the probable error. With one exception (9b), one could be reasonably certain that the result of a single test on any one of the first 15 samples would not be more than 30 minutes in error. In a similar way, the values for the samples requiring from 7 to 8 hours for reduction range from approximately 40 to 95 minutes.

The results of these experiments demonstrate clearly the reliability of the methylene-blue reduction test. If there is a 30 to 1 chance that the results of a single test are not more than 10 to 30 minutes in error, there is little likelihood that the sanitary quality of a sample of milk will be grossly misjudged when the results are interpreted.

In calculating the correlation between the reduction time and the plate count the results of samples 5a, b, d, e, and 14a, b, d, e, were eliminated because of the obvious effect of adding varying quantities of methylene blue. A general survey and comparison of the figures in Table 1 showing plate counts and reduction times indicate a fair, though by no means perfect, correlation between the two tests. The coefficient of correlation was found to be -0.82 ± 0.48 . The agreement may be considered good and corroborates the foregoing conclusion as to the reliability of the methylene-blue test.

EFFECT OF MODIFICATIONS OF THE REDUCTASE TEST

By the elimination of many technicalities the methylene-blue reduction test has been very successfully adapted for field conditions and for use in small dairy plants. In the following tables data are presented to show the effect of some of the modifications on the reliability of the test.

BACTERIOLOGICALLY STERILE GLASSWARE

In Table 2 are presented the results of 103 tests on the same sample of milk, in 52 of which the test tubes and pipettes were subjected to boiling water for 15 minutes, and in the remaining 51 tests bacteriologically sterile glassware was used. The average reduction time for the tests in which bacteriologically sterile glassware was used was 257 minutes, as compared with 241 minutes for the other. The question immediately arises whether the difference of 16 minutes is within the limits of normal variation of the test.

In Table 2 and succeeding tables the actual difference between two average reduction times has been divided by the probable error of the difference; if the quotient exceeds 3.2, it is fairly safe to conclude that the modification of the method introduces a measurable variation. Table 2 shows that when the actual difference of 16 minutes is divided by the probable error of the difference a quotient of 6.4 is obtained. This indicates that the use of glassware which has been boiled 15 minutes introduced a measurable error into the results for this sample of milk. It does not necessarily follow, however, that statistical significance and practical significance are coincident.

TABLE 2.—*The effect of using bacteriologically sterile glassware and "practically sterile" glassware on the results of the methylene-blue reduction test in the examination of milk*

Sample No.	Tests	Method of sterilizing glassware	Average reduction time	Difference divided by the probable error of the difference
	<i>Number</i>		<i>Minutes</i>	
19a.....	52	Boiling water, 15 minutes.....	241±2.2	} 6.4
19b.....	51	Autoclave, 20 pounds pressure, 30 minutes.....	257±1.2	

METHODS OF MEASURING MILK

Table 3 shows the results of experiments on two samples of milk in which various methods of measuring the sample were compared. In the first sample the tests measured by means of graduated test tubes and with a 10 c. c. cup gave an average reduction time of 52 and 36 minutes, respectively. The difference is four times the probable error of the difference.

The second sample was measured by three methods and resulted in an average reduction time of 232 minutes for the samples measured in a graduated test tube, 209 minutes for the 10 c. c. cup method, and 219 minutes for those measured with a 10 c. c. pipette. Here again the result with the cup method deviates from the graduated test tube method by an amount equal to 5.4 times the probable error.

The difference between the pipette and the graduated test tube methods (13 minutes) is but slightly more than the probable error of the difference, and hence may be regarded as of no significance.

TABLE 3.—*The effect of various methods of measuring the 10 c. c. sample of milk used on the results of the methylene-blue reduction test*

Sample No.	Plate count per cubic centimeter	Tests	Methods of measuring milk	Average reduction time	Difference divided by the probable error of the difference when using—	
					10 c. c. cup	10 c. c. pipette
		<i>Number</i>		<i>Minutes</i>		
1-----	300,000	50	Graduated test tube.....	52	4.0	-----
		50	10 c. c. cup.....	36		
		50	Graduated test tube.....	232	5.4	1.2
2-----	200,000	50	10 c. c. cup.....	209		
		48	10 c. c. pipette.....	219	-----	.9

STANDARD METHYLENE-BLUE TABLETS

Two solutions of methylene blue were prepared: (1) By dissolving a standard methylene-blue tablet in 200 c. c. of sterile water; and (2) by making a 1 per cent aqueous solution from a carefully weighed sample of the powder and adding 1 c. c. of this solution to 199 c. c. of sterile water. For convenience the latter was called the laboratory solution and the former the tablet solution. These two solutions were tried in parallel experiments on two samples of milk, one of which had a low bacterial count (14,000 per cubic centimeter) and the other a high bacterial count (3,000,000 per cubic centimeter). Table 4 shows that the difference between the average reduction times for the two solutions was only 13 minutes in the milk with low bacterial count and only 2 minutes in the milk containing 3,000,000 bacteria per cubic centimeter. These differences are not significant.

TABLE 4.—*A comparison between the use of a methylene-blue solution prepared in the laboratory and one prepared from a commercial tablet in making the reduction test on milk*

Sample No.	Plate count per cubic centimeter	Tests	Kind of methylene-blue solution used	Average reduction time	Difference divided by the probable error of the difference
		<i>Number</i>		<i>Minutes</i>	
1-----	14,000	50	Laboratory solution.....	420±13.8	0.6
		50	From tablet.....	433±14.5	
		50	Laboratory solution.....	108±1.2	1.2
2-----	3,000,000	50	From tablet.....	106±1.2	

INACCURACIES IN MEASURING THE DYE SOLUTION

The experiments reported in Table 5 illustrate the effect of adding varying quantities of the methylene-blue solution to the milk. The purpose was to determine the effect of careless measuring or the use of inaccurate pipettes. Two samples of milk were used, one of which gave a plate count of 4,000,000 and the other 19,000 per cubic centimeter. One hundred tests were made on each sample, using varying

amounts of the dye solution in each of five series of 20 tests each. To each tube of a series 0.5, 0.8, 1.0, 1.2, or 1.5 c. c. of methylene-blue solution were added. With both samples the reduction time increased directly as the quantity of the dye solution was increased.

In measuring 1 c. c. of methylene blue it is very unlikely that an error of 0.5 c. c. would result from careless technic or the use of a faulty pipette; however, an error of 0.2 c. c. might easily occur from either of these sources.

The results of these experiments indicate very clearly that the amount of methylene blue used has a direct bearing on the time required for reduction. Gross error in measuring the dye solution will introduce a noticeable effect upon the results of the test and should not be tolerated. Some of the commercial outfits for making this test are equipped with an eye dropper for measuring the dye solution. The relatively large bore of the short tube and the correspondingly wide meniscus are not conducive to accurate measuring. It is doubtful if these should be used.

Attention is called to the fact that the addition of less than 1 c. c. of dye solution introduced greater variation than when more than the standard amount was used; also that the milk with the shorter reduction time (sample 1) was more noticeably affected by errors in measuring the dye than the other sample.

The maximum deviation in the average reduction time resulting from varying the amount of dye solution was 63 and 52 minutes, respectively, for samples 1 and 2. Although these values are probably not of sufficient magnitude to seriously impair interpretation of the test, nevertheless they indicate that care should be exercised in measuring the methylene-blue solution.

TABLE 5.—*The effect of adding varying quantities of methylene blue on the results of the reduction test on milk*

Sample No.	Plate count per cubic centimeter	Tests	Amount of methylene-blue solution used	Average reduction time	Difference divided by the probable error of the difference
		Number	c. c.	Minutes	
1.-----	4,000,000	20	0.5	72±0.7	13.1
		20	.8	102±1.4	4.5
		20	1.0	119±3.5	-----
		20	1.2	129±1.8	2.5
		20	1.5	135±1.7	4.1
		20	.5	374±4.1	5.9
2.-----	19,000	20	.8	397±2.9	2.2
		20	1.0	408±4.0	-----
		20	1.2	419±5.1	1.7
		20	1.5	426±4.7	2.9

SUMMARY AND CONCLUSIONS

The data presented in this paper confirm the general belief among laboratory technicians that methylene-blue reduction tests are characterized by a low degree of variability. When a large number of tests (75-100) were made on each of 19 samples of milk, it was found that the maximum and minimum reduction time for each sample were relatively close together. If the 19 samples of milk had been classified according to the method usually employed in this test, there would

have been 8 in class 1 (reduction time more than 330 minutes), 5 in class 2 (reduction time between 120 and 330 minutes), and 6 in class 3 (reduction time between 20 and 120 minutes).

The values for 3.2 times the probable error for the class 3 samples ranged from ± 2 to ± 17 minutes. One could be reasonably certain that the results of a single test on most of these class 3 samples would not be more than 10 to 15 minutes in error. The limits of a similar degree of reliability for the milk in class 2 were approximately ± 30 minutes, although several samples were far less variable and one was more variable than this would indicate. Likewise, one could be reasonably certain that the reduction time of class 1 milk would not be more than ± 30 minutes in error, providing it did not require more than seven hours for reduction to take place. Of the class 1 samples requiring approximately 7 and 8 hours for reduction, the limits of certainty of the test were 45 and 70 minutes, respectively.

The data also show that the various modifications designed to simplify the test did not seriously impair its accuracy. For example, the difference in the average reduction time for tests in which the sample was measured with a 10 c. c. pipette and a 10 c. c. standard cup was 10 ± 10 minutes; when graduated test tubes were used instead of pipettes the average reduction time was increased 13 ± 10 minutes. The use of glassware that had been only partially sterilized by boiling lowered the reduction time 16 ± 2.5 . Parallel tests using a methylene-blue solution prepared from a tablet and one prepared from the powdered dye gave a difference of 13 ± 20 minutes on milk requiring 7 hours for reduction, and 2 ± 1.7 minutes on another sample of milk in which the reduction time was a little less than 2 hours.

The results of experiments in which varying amounts of dye solution were added indicate that the reduction time is directly related to the amount of dye added. When the inaccuracies of measuring the dye were within the limits of 1 ± 0.2 c. c., an error of 10 to 15 minutes was introduced in the results. However, errors of 0.5 c. c. in measuring the methylene-blue solution introduced variations of 30 to 47 minutes.

The direct relationship between reduction time and the amount of dye solution added indicates that gross errors in measuring would introduce variations of sufficient magnitude to impair the accuracy of the test. Since there is no particular end gained by careless measuring or the use of inaccurate measuring devices, and since the amount of methylene blue added has such a direct bearing on the results obtained, it is advisable to use care in measuring the dye.

When the data are treated statistically, it is found that the limits of normal variation are so close together that even errors of a relatively few minutes are well outside the expected limits of error of the reductase test. Even though an error of 10 to 15 minutes may be significant from a statistical point of view, variations of this magnitude are well within the demands for accuracy in the routine analysis of milk. Many of the modifications have increased the practicability of the test and have made it applicable for use in small plants. The results of these experiments indicate that the simplified methods of making the reductase test which have so greatly enlarged its field of adaptability do not seriously affect the interpretative value of the test.

SEX DIFFERENCES IN THE NORMAL GROWTH RATE OF CHICKS¹

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There is frequent need for normal growth standards in certain phases of chick nutrition work. Among such needs is a standard for the reduction of the population of a lot or group to a single standard for comparison with other lots identically treated but varying in the distribution of the sexes within the lot. Several expedients have been used to compensate for this variation. If the lots chance to be made up of equal numbers of either sex, comparisons may be made directly, but in the case of experimental work with very young chicks, since the sexes can not be differentiated at hatching time, the random selection of the group can not insure equality of numbers of either sex chosen.

Three ways of correcting for the resulting discrepancy in the numbers suggest themselves. The growth data of a group may be corrected to a basis of all males, to a basis of all females, or to the basis of an even division of the sexes. The choice of the method to be used will be a matter of individual preference. The element lacking is the conversion factor. In the interpretation of some work done at the Nebraska station it was thought desirable to attempt the determination of such a factor for Single Comb White Leghorn chicks. The growth records of several hundred chicks from 1 week to 9 weeks of age were available as a result of numerous projects involving them as subjects.

Some of these experiments were designed to produce nutritional failure with death or subnormal growth as the direct result. Such birds were obviously unsuited to the determination of a normal conversion factor, so of course the data on such lots were not included. It was, however, possible to fix an arbitrary standard of growth, the one determined upon being an average weight of 550 gm. for the lot at the age of 9 weeks, before including that lot in the tabulation. In this way lots of birds which had, on the whole, made normal growth or better were used, and the records of lots making subnormal growth were excluded from the calculations.

Thus while it is true that all the chicks were not raised on the same ration, they were all raised on rations which promoted satisfactory growth. The brooding conditions under which the different lots of chicks were raised were good, as is indicated by low mortality in all lots chosen. The chicks were raised in a steam-heated brooder house divided into several runs 4 feet wide and 13 feet long. From 25 to 35 chicks were brooded in each of these pens. Some, but not all, of the lots had access to screened inclosures 4 feet square on the south side of the brooder house when exposure to direct sunshine was desired. The floor was of concrete with pine shavings serving as litter. Electrically heated hovers were used for temperature control. The all-mash method of feeding was employed, no scratch feed being given. Tap water was before the birds at all times.

¹ Received for publication Nov. 22, 1929; issued May, 1930. Contribution from the Departments of Agricultural Chemistry and Poultry Husbandry. Published with the permission of the director of the Nebraska Agricultural Experiment Station as paper No. 84, Journal Series. This paper was read before the division of biological chemistry, American Chemical Society, Minneapolis, Minn., Sept. 10, 1929.

Hendricks, Lee, and Titus² suggested that the slightly lower growth secured in their work for the first 7 to 10 weeks might have been due to rearing the chicks on concrete floors and under conditions of partial confinement. In the work here reported the chicks were also reared on concrete floors. They had no access to soil in the yards, and each lot had a maximum floor space of 68 square feet. Since they made satisfactory growth under these conditions, it would seem that other factors than close confinement to concrete yards must have been responsible for the lower growth reported by the above writers.

When the selections from the record were complete it was found that growth data on 397 males and 403 females were available for the purposes of the study. The chicks were leg-banded at hatching time. When it became possible to distinguish the sex of the bird this fact was noted on the record, so that the individual sex and weight records were complete. Individual weighing were made at 1, 3, 5, 7, and 9 weeks of age. These data were then analyzed statistically at the five ages for each sex, and the mean, the probable error of the mean, the standard deviation together with its probable error, the probable error of the weight of a single chick, and the coefficient of variability and its probable error were all found by the method outlined by Davenport.³ The results of this analysis are shown in Table 1.

TABLE 1.—Mean weights of chicks at different ages

FEMALES				
Age in weeks	Mean weight	Standard deviation	Probable error per single chick	Coefficient of variability
	<i>Grams</i>			<i>Per cent</i>
1.....	57.5±0.28	8.47±0.20	5.71	14.72±0.36
3.....	128.7±.80	23.89±.57	16.11	18.56±.46
5.....	242.5±1.64	48.96±1.16	33.02	20.18±.50
7.....	380.1±2.57	76.56±1.62	51.64	20.14±.50
9.....	532.0±3.43	102.00±2.40	68.79	19.17±.47
MALES				
1.....	60.4±0.31	9.09±0.22	6.13	15.05±0.37
3.....	137.5±.99	29.10±.70	19.63	21.16±.52
5.....	259.5±1.95	57.73±1.38	38.94	22.25±.56
7.....	418.7±2.96	87.42±2.09	58.96	20.87±.52
9.....	606.4±4.00	118.16±2.83	79.73	19.49±.48

The mean female weight can now be compared with the mean male weight, and the differences between the means at the five ages, together with the probable error of these differences, can be calculated, as has been done in Table 2. The ratio of these differences to their probable errors, then, indicates the significance of the differences. It will be noted that when dealing with 400 males and 400 females there is a significant difference between the mean male and mean female weights from the first weighing to the last. This bears out the conclusion of Jull,⁴ who stated that much larger numbers of

² HENDRICKS, W. A., LEE, A. R., and TITUS, H. W. EARLY GROWTH OF WHITE LEGHORNS. Poultry Sci. 8: 315-327. 1929.

³ DAVENPORT, E. PRINCIPLES OF BREEDING; A TREATISE ON TREMMATOLOGY OR THE PRINCIPLES AND PRACTICES INVOLVED IN THE ECONOMIC IMPROVEMENT OF DOMESTICATED ANIMALS AND PLANTS. (With appendix by H. L. Rietz.) 727 p. illus. Boston, New York, [etc.]. 1907.

⁴ JULL, M. A. DIFFERENTIAL SEX GROWTH CURVES IN BARRED PLYMOUTH CHICKS. Sci. Agr. 4: 58-65, illus. 1923.

birds than he used in his study would have to be employed to show a significant difference in mean male and female weight before the eighth week of life. Two other columns are included in Table 2, the first comparing the female weight as a percentage of the male weight, the second showing the ratio of the male to the female weight.

TABLE 2.—*Comparison of the mean weights of male and female chicks at different ages*

Age in weeks	Difference between means	Ratio of difference to its probable error	Mean female weight as per cent of mean male weight	Mean male weight divided by mean female weight
	<i>Grams</i>			
1.....	2.9±0.42	6.9	95.21	1.051
3.....	8.8±1.28	6.9	93.61	1.069
5.....	17.0±2.55	6.7	93.44	1.070
7.....	38.6±3.92	9.8	90.78	1.102
9.....	74.4±5.28	14.1	87.72	1.140

While there is a significant difference between the mean male and female weights at 1 week of age, it can be readily appreciated that the sex of the week-old chick can not be inferred from its weight, for the magnitude of the probable error of the weight of a single chick, or of the coefficient of variability, precludes the possibility of such an inference.

The point to be observed is that the mean weight of a group of chicks at 9 weeks of age is influenced by the number of males in proportion to the number of females. If the distribution between the sexes is not equal, discrepancies will arise when lots are compared on the basis of the mean weight of the lots without making a correction in the proper sense. It would seem feasible to make such a correction by using the factor of the ratio of male to female weight as found in a study of the growth rates of 400 individuals of each sex. As noted in Table 2, this ratio is, at 9 weeks of age, female weight times 1.140 equals male weight. This is the procedure followed in correcting the weight of the females of a lot to the basis of the weight of all males. It can be applied individually to convert the weight of each female to a male basis, or the average female weight can be converted to a male basis, and from this value, together with the actual weights of the males involved, the whole lot can be brought over to the basis of all males. That the distribution of sexes in the lot is a factor is shown by Table 3.

TABLE 3.—*Effect of unequal division of sexes on weight averages in flocks of 40 chicks*

Males	Females	Average weight of 40 chicks	Males	Females	Average weight of 40 chicks
<i>Number</i>	<i>Number</i>	<i>Grams</i>	<i>Number</i>	<i>Number</i>	<i>Grams</i>
0	40	532	25	15	578
5	35	541	30	10	588
10	30	550	35	5	597
15	25	560	40	0	608
20	20	569			

Take a lot of 40 birds and assume that the mean weight of the males is 606 gm. and that of the females 532 gm. Varying the number of males in this hypothetical lot from 0 to 40, while the number of females is varied conversely, will bring out the discrepancies that arise from a failure to take this distribution into account.

If the average weight is calculated upon the total weight of all the males and females, divided by 40, the above table is obtained. This illustrates the fact that the average or mean weight of the lot would depend on the ratio of male to female birds in the lot. However, by the use of the factors found in Table 2, it is possible to compare a lot with one proportion of males to a lot having another proportion. By the use of the factor the lot may be converted to the basis of all males, in which case the mean weight would be 606 gm., all females, in which case the mean weight would be 532 gm., or half males and half females, in which case the mean weight would be 569 gm. Direct comparison is thus obtained regardless of the basis selected. If the mean weight of each lot is to be used as one of the criteria the lots must be brought to a uniform basis before comparison is attempted.

SUMMARY

The growth data of 397 male and 403 female Single Comb White Leghorn chicks from 1 to 9 weeks of age have been subjected to a statistical examination. Differences in the mean weight of male and female chicks are significant at 1 week of age, and the significance increases thereafter.

A factor is suggested for use in bringing lots with different numbers of male and female chicks to a common basis for the purpose of comparison.

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A STUDY OF THE STRUCTURE OF SUGAR BEETS IN RELATION TO SUGAR CONTENT AND TYPE¹

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INTRODUCTION

In order to recognize a plant type and to learn its inherent possibilities it is necessary to delimit it sharply. This has been done for most cultivated plants, but beet fields often show even within a limited area plants of most diversified root and foliage characters. The system of mass selection used in beet-breeding work is largely responsible for this diversity. The commercial beets have been bred for performance, and whenever the performance of certain seed lots sank below a certain level new "families" were introduced. Perhaps the principal reason for not employing pure-line selection methods has been the common belief in the protandry of the beet flower. Recent investigations, however, have greatly weakened the foundation for this concept, for it is now an established fact that close fertilization in beet flowers is theoretically possible (*1*),³ and it seems probable that failures in pure-line work may have been due to self-incompatibility or certain other factors; but that this condition may not necessarily be a stumbling block has been suggested by many workers (Munerati, Vilmorin, Nilsson), who vigorously combat the general commercial viewpoint that pure-line methods have no applicability in sugar-beet breeding work.

Most of the morphologically uniform beet selections in this country were the outcome of the indefatigable labors of W. W. Tracy, formerly at the sugar-plant breeding station of the United States Department of Agriculture at Fort Collins, Colo. Tracy's lines were originally selected out of ordinary commercial stock. All samples were first bred by isolating roots individually from two to four generations. After this number of generations of individual isolations of single roots, the samples were in many cases thrown into group isolations. The mother beets of the various strains were grown separately under good conditions of isolation in order to prevent cross pollination with other beets.

The work reported in this paper is an anatomical study of a number of Tracy's selections for the purpose of finding characters that would aid in determining the purity of a type, in delimiting it more sharply,

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² The writer is under obligation to W. W. Tracy and D. W. Koonce for growing the material used in this study, to S. F. Sherwood and Mrs. Ruth C. Starrett for the sugar analyses, and to Mrs. E. Artschwager for making the drawings.

³ Reference is made by number (*italic*) to "Literature cited," p. 914.

and in testing on a larger scale than has hitherto been done the various theories of a possible connection between anatomical structure and sugar content.

Although the strains examined were far more uniform than ordinary commercial stock, they do not yet constitute pure lines, and many of the discrepancies in the anatomical characterization of the types are the expression of different line impurities.

MATERIALS AND METHODS

The material studied in 1927 consisted of 700 beets taken from 30 of Tracy's selections. A description of each selection is given in Table 1. The beets were harvested during the first week in October and were analyzed and examined directly in the field laboratory. The beets tested rather low because the season was in general unfavorable for high sugar production and the beets were harvested two weeks in advance of the regular digging. The unevenness in percentage of sucrose was partly the result of leaf-spot infection, from which some plants recovered earlier than others and were able to store a larger amount of sugar. In 1928 only 23 of the original 30 selections were available for study, but the material was grown in two places, at Fort Collins and Fort Lewis, Colo. One thousand beets were harvested the second week in October and were carefully packed and shipped to Washington, D. C., for further study.

The data were taken on cross sections through the neck region of the beet. This region appears to be the best suited for this study, and a number of earlier investigations have been based on it. But even here the outline is not always symmetrical, which makes it difficult to obtain correct ring counts and decreases the accuracy of the measurements. The total diameter of the beet is the mean of the short and long diameters wherever the circumference is not a circle. This applies also to the measurements of the first ring and the central core. The bundles of the vascular rings were counted in stained sections. The topped beet was placed with the neck region in a shallow dish containing Delafield's haematoxylin. The dish with the beet was then transferred to a bell jar, from which the air was subsequently exhausted. After a few minutes the beet was removed from the jar and thin slices were cut, usually with the aid of a slicer. These were examined on an opal glass plate with bottom illumination. The sections appeared white, except for the xylem of the bundles, which stood out a bright purple against the colorless background. The size of the interzonal parenchyma cells was determined by projecting a slide on a white screen and counting the cells of a given area.

The photographs of the beet cross sections are from fresh unstained material taken on panchromatic plates. The central cores were stained with phloroglucin and hydrochloric acid in order to obtain the greatest possible contrast between vascular tissue and parenchyma. The drawings of the vascular bundles are from photomicrographs taken directly on bromide paper. All other photomicrographs were taken on Wratten M plates with B-58 and E-22 filters used singly or in combination.

TABLE 1.—*Description of the 30 sugar-beet selections studied*
[Selections listed in numerical order]

Selection No.	Plant		Foliage		
	Type	Size	Number of leaves	Size	Spread of foliage
150-24	Uniform	Medium	Somewhat large	Medium	Semierect.
193R-25	do	Somewhat small	Somewhat small	Somewhat large	Erect.
554-24	Medium	do	Very large	Somewhat small	Spreading.
600S-25	Uniform	Somewhat large	Small	Medium	Do.
730-24	Medium	Large	do	Somewhat large	Semierect.
898-24	Uniform	Small	Large	do	Decidedly spread- ing.
992S-25	do	Large	Somewhat small	Medium	Semierect.
1161-24	do	Medium	Somewhat large	Somewhat large	Do.
1228S-25	do	Somewhat large	Small	Small	Spreading.
1293S-25	Unlike	Somewhat small	Medium	Medium	Somewhat spread- ing.
1427-24	Uniform	Somewhat large	Somewhat small	do	Semispreading.
1569-24	Very uniform	Small	Small	Small	Spreading.
1591-24	Uniform	Large	Somewhat large	Medium	Semierect.
1612-24	do	do	do	do	Somewhat spread- ing.
1818S-25	do	Medium	do	do	Semierect.
1845S-25	Medium	do	Medium	Small	Somewhat spread- ing.
2287-24	Uniform	Large	Small	Decidedly large	Erect.
2310S-25	do	Decidedly large	Medium	Medium	Do.
2346S-25	Unlike	Medium	Somewhat small	Somewhat large	Somewhat spread- ing.
2445S-25	Uniform	do	Large	do	Semierect.
3403-24	Unlike	do	do	Somewhat large	Do.
3956-24	Medium	Large	Somewhat small	Decidedly small	Very erect.
4545-24	Uniform	do	Small	Large	Erect.
4621-22	Unlike	do	Medium	Small	Somewhat spread- ing.
5656-24	Medium	Medium	Somewhat small	Large	Erect.
5942-24	Uniform	do	Somewhat large	Somewhat small	Semierect.
6077-24	do	Large	do	do	Somewhat spread- ing.
7794-24	Medium	Medium	Large	Medium	Semierect.
8146-24	Uniform	Somewhat large	Somewhat small	Large	Spreading.
8753-24	do	Medium	Large	Medium	Semierect.

Selection No.	Root				
	Size	Shape	Length	Fullness	Taproot
150-24	Medium	Top	Slender, short	Semidepressed	Large.
193R-25	Somewhat small	Parsnip-top	do	Depressed	Decidedly large.
554-24	Small	do	Slender	do	Very large.
600S-25	Decidedly large	Mangel-top	Slender, short	Full	Large.
730-24	Small	Parsnip	Slender	Very full	Small.
898-24	Somewhat large	do	Slender, short	Decidedly full	Do.
992S-25	Decidedly small	Top	Slender	Full	Medium.
1161-24	Small	do	Slender, short	Semidepressed	Small.
1228S-25	do	do	Decidedly short	Full	Very small.
1293S-25	do	do	Short	Decidedly depressed.	Large.
1427-24	Large	do	Slender	Somewhat full	Small.
1569-24	Small	Parsnip	Slender, short	Very full	Somewhat small.
1591-24	Large	Top	Slender	Full	Small.
1612-24	do	do	do	do	Very small.
1818S-25	Medium	Turnip-parsnip	Short	Depressed	Very large.
1845S-25	do	Top	Slender, short	do	Somewhat large.
2287-24	Very large	Mangel-top	Slender	Full	Very small.
2310S-25	Decidedly large	do	Very slender	Semidepressed	Somewhat large.
2346S-25	Medium	Top	Slender, short	Somewhat full	Somewhat small.
2445S-25	do	Mangel-top	do	Very full	Very small.
3403-24	Somewhat large	Top	do	Semidepressed	Medium.
3956-24	Medium	Parsnip	Short	do	Do.
4545-24	Somewhat small	Top	Very slender	Depressed	Very large.
4621-22	Small	do	Decidedly slender	do	Do.
5656-24	Medium	do	Slender, short	Full	Medium.
5942-24	do	do	Short	Very full	Very small.
6077-24	Somewhat large	Mangel-top	do	Full	Small.
7794-24	Medium	Top	Decidedly slender	Semidepressed	Medium.
8146-24	Large	Parsnip	Short	Full	Small.
8753-24	Medium	Top	do	Semidepressed	Medium.

The absence of detailed figures in the published reports makes it impossible for other workers in the field to test the results, and the reader is deprived of the opportunity to check the conclusions of the writer or to make his own deductions. For this reason the results of the important morphological and anatomical measurements made in this study are given in detail for 20 of the 30 selections studied. (Table 2.) The mean, standard deviation, and coefficient of variability of some of the important characters are also shown. (Table 3.)

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*

[Diameters were measured in the neck region (the smooth zone just below the leaf scars). Selections listed in numerical order]

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
	Per cent	Grams	Mm.	Mm.	Mm.	Number		Mm.	Number	
550-24; 1927-----	11.3	1,193	110	31	10	11	2.0	8	160	Absent.
	11.4	795	90	29	7	10	2.2	6	100	Do.
	11.7	907	95	27	6	10	1.9	6	178	Present.
	11.8	907	90	29	8	9	1.8	8	188	Do.
	12.1	907	103	23	6.5	10	2.0	8	200	Absent.
	12.7	907	90	20	5	9	2.1	7	200	Present.
	13.0	907	85	25	7	8	2.1	7	120	Absent.
	13.1	738	85	20	5	9	2.2	6	200	Present.
	13.5	680	80	26	7	9	2.2	8	120	Absent.
	13.5	958	103	30	9	10	2.0	8	130	Do.
	13.7	1,079	103	26	6.5	10	1.9	8	154	Present.
	13.8	568	80	20	3.5	9	2.3	6	154	Absent.
	13.9	1,022	93	22	5	13	2.4	7	193	Do.
	14.0	596	80	20	4	9	2.4	5	169	Do.
	14.1	852	93	20	5	12	2.5	7	169	Do.
	14.3	680	80	21	5.5	11	2.7	6	170	Do.
	14.5	738	88	23	5	11	2.3	6	129	Do.
	14.5	795	85	25	5	10	2.3	7.5	110	Do.
	14.7	1,079	100	23	4	10	2.2	7	168	Do.
	14.7	795	90	23	4	11	2.6	7	142	Present.
	15.1	680	80	24	6	9	2.3	5.5	138	Absent.
	15.1	738	80	21	6	11	2.7	5	154	Do.
	15.2	938	100	20	3.5	11	2.3	7.5	171	Do.
	15.4	598	75	20	4.5	9	2.2	6	154	Do.
	15.8	680	88	18	3	13	2.7	6	130	Do.
1928-----	15.4	680	90	20	3	10	2.1	-----	170	-----
	16.1	1,052	105	22	5	9	1.8	-----	140	Present.
	16.6	738	93	23	4.5	9	1.9	-----	160	-----
	16.8	738	83	20	4	10	2.2	-----	130	-----
	17.0	1,022	97	20	2.5	11	2.1	-----	190	Do.
	17.4	1,022	72	22	3	11	2.1	-----	150	-----
	17.4	882	90	22	3	10	2.2	-----	120	Do.
	17.5	738	90	21	4	14	2.6	-----	160	-----
	17.6	738	85	23	4.5	10	2.1	-----	150	-----
	17.6	680	80	23	4.5	9	2.2	-----	150	-----
	17.8	1,079	92	19	3	10	1.9	-----	150	Do.
	17.8	768	96	16	4	12	2.3	-----	130	Do.
	18.2	769	85	21	5	11	2.4	-----	130	-----
	18.2	768	92	19	3	11	2.4	-----	140	-----
	18.5	680	91	18	3	10	2.2	-----	140	Absent.
	18.6	768	92	23	5	12	2.3	-----	140	-----
	18.8	1,022	100	16	2.5	10	1.9	-----	200	-----
	19.8	710	90	22	5	10	2.2	-----	190	Do.
	20.2	907	95	17	2.5	10	2.0	-----	180	Do.
	20.4	598	79	15	2	12	2.7	-----	200	Do.
	(*)	710	85	18	3.5	12	2.4	-----	-----	-----
	(*)	882	90	24	5	11	1.9	-----	-----	-----
	(*)	483	70	14	2.5	10	2.3	-----	-----	-----
	(*)	795	95	23	4.5	11	2.0	-----	-----	-----
	(*)	907	95	16	2	13	2.2	-----	-----	-----

* Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
554-24:	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
1927-----	11.0	1,477	103	19	2.5	10	1.9	9	153	Present.
	11.2	1,306	103	23	5	11	2.1	8	110	Do.
	11.4	625	75	22	6	10	2.6	4.5	185	Do.
	11.7	625	70	11	1.5	10	2.5	3.5	133	Do.
	12.2	907	85	20	4	9	2.1	6	172	Do.
	12.3	958	85	22	4	9	2.3	7	191	Do.
	12.4	738	78	17	2.5	11	2.3	7	161	Do.
	12.6	852	90	15	1.5	10	2.1	5	218	Do.
	13.4	907	93	18	2.5	9	1.9	7	166	Present.
	13.8	511	75	12	2	9	2.6	5	227	Do.
	13.8	1,079	95	17	3.5	8	1.7	7	170	Do.
	13.9	958	95	18	3	8	1.7	7	200	Do.
	14.1	852	90	23	2.5	8	1.7	7	142	Present.
	14.1	738	85	13	1.5	10	2.3	5	180	Absent.
	14.1	1,134	100	25	4	11	2.1	5	144	Do.
	14.2	852	83	16	2.5	9	2.2	6	158	Present.
	14.2	680	80	16	3	11	3.0	4	133	Absent.
	14.3	795	90	20	4	10	2.1	7	200	Do.
	14.5	680	80	20	4	8	2.1	6	161	Do.
	14.5	795	90	20	3	9	2.0	5	141	Do.
	14.8	568	73	19	1.5	11	2.3	4	154	Do.
	14.9	795	75	17	3	12	2.3	5	133	Do.
	15.1	958	93	17	3	10	2.0	7.5	181	Do.
	16.1	625	80	18	2	10	2.4	4	161	Present.
1928-----	16.2	511	70	15	2.5	10	3.0	4	158	Absent.
	16.2	680	76	14	2	9	2.3		160	Do.
	16.2	795	77	15	2	9	2.3		160	Do.
	16.8	680	85	16	2	11	2.2		160	Do.
	17.0	937	76	16	5	13	3.0		160	Present.
	17.2	795	83	14	2.5	9	2.0		160	Absent.
	17.4	907	90	14	2	11	2.2		150	Do.
	17.6	937	86	15	2.5	12	2.6		160	Do.
	17.8	882	86	15	2	11	2.4		180	Do.
	17.8	1,250	90	18	2	12	2.4			
	17.8	795	80	15	2	13	2.9			
	18.0	680	77	18	4	9	2.3		170	Do.
	18.2	1,052	83	17	3.5	11	2.7		160	Do.
	18.2	937	82	15	2.5	10	2.2			
	19.0	882	83	11	1.5	12	2.6			
	19.2	680	74	22	6	10	2.4		170	Do.
	19.4	852	85	14	2	12	2.6		160	Present.
	19.4	710	83	15	2	11	2.6			
	19.8	825	80	14	3	12	2.9		210	Do.
	20.2	907	85	18	3.5	13	2.9		150	
	20.4	710	80	15	2	11	2.5			
	(*)	768	79	16	1.5	9	2.3			
	(*)	625	76	11	2	12	2.9			
	(*)	825	80	14	2	10	2.2			
	(*)	1,109	83	17	2	11	2.4			
	(*)	598	72	19	2	8	2.3			
600s-25:										
1927-----	9.0	568	83	24	6	8	1.9	7	157	Do.
	10.3	958	100	21	5	10	2.1	7	170	Do.
	10.8	852	98	21	5	12	2.4	7	118	Absent.
	10.8	758	85	20	3	9	2.1	5	133	Present.
	11.1	958	90	28	7.5	9	1.9	7	142	Absent.
	11.1	958	103	21	3	11	2.1	6	145	Do.
	11.3	680	93	26	9	9	2.0	5	139	Do.
	11.5	1,022	105	22	4	13	2.6	7	100	Do.
	11.7	852	95	28	9	8	1.9	7	100	Present.
	12.0	958	103	25	5.5	10	2.0	7	133	Absent.
	12.2	1,250	105	22	4	11	2.0	6	130	Present.
	12.2	1,022	110	24	8	10	1.7	6	110	Absent.
	12.2	1,022	95	17	3	13	2.5	7	110	Present.
	12.3	625	78	17	4	10	2.4	7	100	Absent.
	12.3	1,193	103	27	9	10	2.4	9	150	Do.
	12.4	907	90	16	3	11	2.4	6	150	Do.
	12.4	852	98	16	3	11	2.7	5	158	Do.
	13.2	1,193	105	22	3	12	2.3	6	100	Present.
	13.3	738	93	30	5	11	2.3	7	180	Do.
	13.5	568	70	22	8	8	2.0	5	142	Absent.

* Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
600s-25—Contd.	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
1927-----										
13.5	1,420	103	25	5.5	11	2.2	8	131	Absent.	
13.8	625	83	20	4.5	10	2.4	7	140	Do.	
13.8	738	85	17	5	12	2.5	6	191	Do.	
14.0	958	103	18	3	12	2.2	7	150	Do.	
14.0	852	85	27	7	9	2.0	7.5	175	Do.	
1928-----										
12.8	738	96	22	6.5	9	1.7	-----	140	Present.	
13.5	1,193	112	24	6	10	1.7	-----	140	Absent.	
13.6	988	102	23	5.5	11	1.9	-----	150		
14.6	907	100	27	7	10	2.0	-----	80		
15.2	937	93	21	7	13	2.3	-----	160		
15.4	1,134	105	23	3.5	11	1.8	-----	130	Present.	
15.4	710	85	17	3.5	11	2.2	-----	150		
15.5	1,022	92	26	7	10	1.8	-----	140	Absent.	
15.6	1,022	99	24	6	14	2.4	-----	140		
15.6	907	86	22	4	9	2.0	-----	110	Do.	
16.0	852	96	20	2.5	11	2.1	-----	100		
16.0	988	83	19	3.5	11	2.1	-----	140		
16.0	937	102	25	6	10	1.7	-----	110		
16.8	1,022	95	22	3.5	10	2.0	-----	130		
16.8	1,052	105	20	3.5	12	2.1	-----	140	Do.	
17.5	1,134	205	19	3.5	12	1.8	-----	210	Do.	
17.5	795	97	14	3	13	2.5	-----	100		
17.6	907	88	25	6	12	2.2	-----	170		
18.0	958	90	17	4	15	2.3	-----	150		
18.0	852	90	19	2.5	10	2.1	-----	150		
(*)	907	95	23	5	10	1.9	-----	-----	Do.	
(*)	1,022	100	22	3.5	11	1.9	-----	-----	Do.	
(*)	907	99	25	6	11	2.2	-----	-----	Do.	
(*)	852	99	20	3	12	2.0	-----	-----	Do.	
(*)	598	85	20	4	11	2.4	-----	-----	Do.	
898-24:										
1927-----										
11.5	1,079	95	21	3	10	2.0	8	121	Do.	
11.8	1,022	98	24	5	11	2.4	7	170	Do.	
12.0	907	95	24	7	10	2.2	6.5	112	Do.	
12.1	1,079	95	17	4	11	2.2	6.5	190	Do.	
12.3	852	80	15	4	10	2.4	6.5	135	Do.	
12.5	1,022	103	25	7	10	2.0	6	144	Do.	
12.6	1,079	95	21	3	12	2.2	7	137	Do.	
12.6	738	80	19	6	12	2.6	6	112	Do.	
12.8	1,306	103	24	7	11	2.1	9	121	Do.	
13.0	958	88	25	8	9	2.1	7	137	Do.	
13.0	680	80	22	9	11	2.6	6	112	Do.	
13.2	625	75	21	4	10	2.4	7	157	Do.	
13.4	1,022	90	24	6	11	2.2	7	137	Do.	
13.5	738	80	17	2	10	2.6	5.5	129	Do.	
13.6	1,079	93	20	2	11	2.3	7	130	Do.	
13.6	852	85	27	10	8	1.9	7	98	Do.	
14.2	958	90	27	11	11	2.3	3.5	137	Do.	
14.3	907	90	24	4	10	2.2	6.5	121	Do.	
14.3	680	83	18	6	11	2.4	5	119	Do.	
14.6	1,079	95	17	2	10	2.1	6	200	Do.	
1928-----										
13.5	825	90	26	7	11	2.44	6	110	Present.	
14.2	765	80	18	3.5	10	2.00	7	89	Absent.	
13.4	907	102	23	8	10	1.85	7	87	Do.	
15.4	935	92	23	5	10	2.04	7	146	Do.	
15.8	985	90	19	5	10	2.00	6.5	106	Do.	
16.0	1,193	104	25	5	11	2.00	7	130	Present.	
16.4	958	90	20	5	12	2.40	7	113	Absent.	
16.4	907	87	17	3	12	2.50	6	140	Do.	
16.5	852	90	16	3	12	2.35	6	140	Do.	
16.5	935	92	19	4	12	2.31	6.5	105	Do.	
17.0	738	80	20	3.5	11	2.60	7	100	Do.	
17.1	680	80	20	3.5	10	2.32	6.5	132	Do.	
17.0	795	85	20	4	11	2.44	6	100	Do.	
17.5	1,134	97	20	4	11	2.07	8	131	Do.	
17.6	795	80	16	4	12	2.35	6	100	Do.	
18.2	985	82	20	5	12	2.40	6	112	Do.	
18.2	825	90	17	5	12	2.35	7	134	Do.	
18.2	710	77	17	4	10	2.08	5	125	Do.	
18.3	852	87	18	5	9	1.91	8	140	Do.	
18.4	907	92	22	6	11	2.07	8	145	Do.	
18.6	958	87	20	5	11	2.11	6	125	Present.	

* Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
898-24—Contd.	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
1928-----	18.6	1,361	100	21	6	12	2.10	7	97	Absent.
	19.0	680	75	17	4	11	2.56	6	146	Do.
	19.3	852	90	18	3.5	11	2.11	7	144	Do.
	19.5	680	80	17	4	12	2.61	6	125	Do.
1161-24:										
1927-----	10.7	625	85	17	4	9	2.1	6.5	166	Do.
	11.1	680	83	15	3	10	2.2	5	142	Present.
	11.3	795	90	19	3	9	2.0	6	166	Absent.
	11.4	958	85	22	6.5	10	2.3	5.5	90	Do.
	11.8	680	85	17	3	10	2.4	5	116	Do.
	12.1	1,590	113	22	6.5	10	1.8	7.5	210	Do.
	12.1	1,134	98	22	6.5	13	2.4	6	133	Present.
	12.4	625	75	17	3.5	9	2.4	7	146	Do.
	12.4	738	85	19	5.5	8	1.9	5	131	Absent.
	12.5	1,079	95	24	6.5	10	2.0	7	133	Do.
	12.7	907	83	21	6	12	2.7	5.5	120	Do.
	12.8	1,250	110	21	5	10	2.0	8	200	Do.
	12.8	680	78	20	7	9	2.4	5	121	Present.
	12.8	852	83	20	4.5	9	2.1	6.5	190	Absent.
	12.9	958	93	25	8	10	2.1	6	142	Present.
	13.1	907	90	19	4	9	1.9	6.5	182	Absent.
	13.2	795	80	23	6	8	1.7	7	170	Do.
	13.2	680	83	19	3	10	2.1	5	141	Present.
	13.3	958	87	20	5	11	2.3	6	163	Do.
	13.3	958	90	21	5	9	2.0	7	161	Do.
	13.5	1,022	95	20	4	9	1.8	8	200	Do.
	13.6	907	93	20	3	9	2.0	4	150	Absent.
	13.8	958	93	19	4	10	2.0	7	133	Present.
	13.9	680	85	15	3	10	2.4	5	200	Absent.
1928-----	15.0	680	80	21	5	10	2.4	6	146	Do.
	15.6	1,022	94	15	3	11	2.2	-----	140	Present.
	16.4	710	85	17	2.5	10	2.1	-----	150	Absent.
	16.6	882	85	16	3	10	2.1	-----	140	Do.
	16.6	541	75	15	2	10	2.4	-----	150	Do.
	17.1	680	83	14	2.5	10	2.0	-----	110	Do.
	17.2	541	78	14	2	9	2.0	-----	140	Do.
	17.8	937	90	17	3	10	1.9	-----	190	Present.
	17.4	852	84	19	3.5	8	2.1	-----	130	Absent.
	17.4	795	87	15	2.5	10	2.0	-----	160	Do.
	17.5	710	77	16	3	10	2.3	-----	140	Do.
	17.7	907	84	13	2	9	1.9	-----	140	Do.
	17.9	710	80	14	2	10	2.0	-----	150	Do.
	18.2	958	86	18	3	10	1.9	-----	130	Do.
	18.2	568	80	14	2	10	2.2	-----	150	Do.
	18.3	795	88	16	2.5	10	1.9	-----	150	Do.
	18.4	568	73	11	1.5	9	2.2	-----	160	Do.
	18.4	680	80	12	2	10	2.3	-----	180	Do.
	18.5	738	85	14	2	10	2.0	-----	150	Do.
	18.8	680	80	15	2.5	9	1.9	-----	140	Do.
	19.4	795	83	15	2.5	9	2.0	-----	120	Do.
(a)	795	85	-----	-----	-----	-----	-----	-----	-----	-----
(a)	625	74	18	4	-----	9	2.1	-----	-----	-----
(a)	483	79	11	2	-----	10	2.3	-----	-----	-----
(a)	458	66	13	2	-----	10	2.6	-----	-----	-----
(a)	483	70	15	3	-----	9	2.1	-----	-----	-----
1228s-25:										
1927-----	9.7	1,079	110	23	5	10	1.8	6	158	Present.
	10.1	1,306	110	23	5	10	1.8	8	118	Do.
	10.5	1,134	105	27	5	9	1.7	7	166	Absent.
	10.6	568	80	23	6	9	2.1	6	200	Do.
	11.4	511	80	20	3.5	9	2.1	5	133	Do.
	11.6	855	90	25	5	9	1.8	7	150	Do.
	11.7	852	90	23	5	9	1.9	6	125	Do.
	11.7	738	95	20	4	10	2.0	5	145	Do.
	12.0	625	90	26	6	9	2.0	6	130	Do.
	12.0	680	90	23	5	8	1.7	7	136	Do.
	12.1	738	100	25	4	8	1.7	7	200	Do.
	12.3	958	90	26	6	10	2.1	7	154	Do.
	12.5	958	95	20	2.5	10	2.1	7	125	Do.
	12.5	958	85	23	5	9	1.8	7	163	Do.
	12.7	852	100	20	3.5	11	2.2	5	125	Do.
	12.8	568	83	23	2.5	9	2.1	6	175	Do.

(a) Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coeff- cient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
1228s-25—Contd.	Per cent	Grams	Mm.	Mm.	Mm.	Number		Mm.	Number	
1927-----	13.0	907	100	23	4	8	1.7	6	133	Absent.
	13.0	1,195	110	25	-----	10	1.8	7	133	Do.
	13.2	1,195	110	24	6	11	2.0	6	145	Present.
	13.2	453	80	23	4.5	7	1.8	6	142	Absent.
	13.3	738	98	23	5	10	2.0	5	118	Do.
	13.8	625	83	20	4	10	2.3	4	166	Do.
	13.9	958	90	21	5	11	2.1	5	140	Do.
	13.9	1,022	100	25	7	9	1.7	6	175	Present.
	14.4	852	85	20	4	10	2.3	6	163	Absent.
1928-----	15.0	710	85	27	5	9	1.98	7	89	
	15.0	852	95	18	3	10	2.00	7	110	
	15.4	795	90	20	3	9	1.87	5	109	
	15.4	710	85	17	3	9	1.73	5	204	
	15.4	680	84	19	4	10	2.13	5	100	
	15.7	882	101	20	3.5	10	1.85	6	175	
	15.8	1,022	91	18	3	10	1.98	6	80	
	15.8	1,022	102	25	5	10	1.80	6.5	96	
	16.0	768	88	16	2	10	2.08	7	87	
	16.0	568	85	20	3	9	2.14	6	132	
	16.2	595	85	20	3	9	1.80	4	96	
	16.2	880	102	21	3.5	9	1.61	4	195	
	16.6	595	85	20	4	9	2.00	4	113	
	16.6	795	95	19	3	9	1.80	6	187	
	17.0	852	92	20	4.5	10	1.89	6	105	
	17.0	795	96	18	3	11	2.00	5	123	
	17.0	880	94	14	2	12	2.40	7	80	
	16.8	670	85	20	4	8	1.54	4	80	
	17.5	680	95	19	3.5	10	2.08	6	105	
	17.6	764	90	23	4.5	10	2.13	8	136	
	17.8	680	83	22	5	9	1.87	6	104	
	17.8	680	80	15	1.5	10	2.13	4	108	
	17.9	795	97	25	4.5	10	1.85	6.5	139	
	18.6	768	90	20	4.5	10	2.08	6	123	
	18.6	738	82	12	1.5	11	2.24	7	139	
1427-24: 1927-----	11.5	1,079	95	21	3	10	2.0	8	141	Do.
	11.8	1,022	98	24	5	11	2.4	7	170	Present.
	12.0	907	95	24	7	10	2.2	6.5	124	Absent.
	12.1	1,079	95	17	4	11	2.2	6.5	118	Do.
	12.3	852	80	15	4	10	2.4	6.5	194	Do.
	12.5	1,022	103	25	7	10	2.0	6	141	Do.
	12.6	1,079	95	21	3	12	2.2	7	147	Do.
	12.6	738	80	19	6	12	2.6	7	124	Present.
	12.8	1,306	103	24	7	11	2.1	9	130	Absent.
	13.0	958	88	25	8	9	2.1	7	153	Do.
	13.0	680	80	22	9	11	2.6	6	159	Do.
	13.2	625	75	21	4	10	2.4	7	182	Do.
	13.4	1,022	90	24	6	11	2.2	7	153	Do.
	13.5	738	80	17	2	10	2.6	5.5	153	Do.
	13.6	1,071	93	20	2	11	2.3	7	141	Present.
	13.6	852	85	27	10	8	1.9	7	130	Absent.
	14.2	958	90	27	11	11	2.3	7.5	159	Do.
	14.3	907	90	24	4	10	2.2	6.5	147	Present.
	14.3	680	83	18	6	11	2.4	5	159	Absent.
	14.6	1,079	95	17	2	10	2.1	6	279	Do.
1928-----	15.4	738	78	13	2	10	2.30	-----	150	
	16.8	568	87	23	4	11	2.10	-----	160	
	16.8	1,192	74	16	2	10	2.40	-----	200	
	16.8	680	69	18	3	11	2.90	-----	200	
	17.0	795	73	13	2	11	2.60	-----	180	
	17.2	598	65	16	2.5	11	2.90	-----	130	
	17.2	710	69	14	2	10	2.50	-----	180	
	17.2	511	68	14	2.5	12	3.30	-----	130	
	17.2	680	76	20	3.5	8	1.90	-----	170	
	17.8	768	75	16	2.5	11	2.50	-----	180	
	17.5	710	80	20	3.5	10	2.20	-----	160	
	17.5	795	75	16	2.5	10	2.50	-----	180	
	17.8	680	73	16	2	10	2.30	-----	140	
	17.8	680	68	12	2	10	2.50	-----	120	
	18.2	768	79	13	3	11	2.30	-----	240	
	18.5	625	75	16	2	11	2.50	-----	100	
	18.6	907	87	18	2	12	2.50	-----	160	
	18.6	795	83	16	2.5	12	2.30	-----	220	

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
1427-24—Contd.	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
1928.....	18.6	680	72	18	5	9	2.30	-----	120	
	18.8	710	80	18	3	11	2.60	-----	170	
	(*)	680	75	19	3	10	2.40	-----	-----	
	(*)	568	68	25	6.5	8	2.10	-----	-----	
	(*)	625	70	16	3	-----	2.80	-----	-----	
	(*)	568	73	22	6	-----	2.60	-----	-----	
	(*)	423	60	11	2	-----	3.00	-----	-----	
1569-24:										
1927.....	9.6	1,134	95	22	6	9	1.7	7	172	Absent.
	9.8	852	83	30	10	9	2.0	4.5	190	Do.
	10.5	625	80	18	5	10	2.2	6	153	Do.
	10.7	680	83	22	6	9	2.3	4	200	Do.
	10.7	738	83	20	4	10	2.7	5	108	Do.
	10.7	958	85	26	10	10	1.9	6	126	Do.
	10.8	795	85	23	5	9	2.2	6	114	Do.
	11.2	738	85	20	5	9	2.2	6	175	Do.
	11.3	1,079	93	22	5.5	10	2.1	5	180	Do.
	11.7	568	80	26	6	11	2.5	5	100	Do.
	11.8	625	78	22	6	9	2.6	5.5	113	Do.
	11.8	795	85	22	6	10	2.4	5	137	Do.
	12.0	680	95	20	5.5	10	1.9	6	144	Do.
	12.0	738	88	20	5	10	2.1	6.5	119	Do.
	12.5	738	95	22	6	10	2.0	5	142	Do.
	12.6	738	88	23	7	11	2.2	6	200	Do.
	12.8	511	68	23	4	9	2.4	5	147	Do.
	12.8	625	88	18	5	10	2.3	5	160	Do.
	12.8	738	83	23	7	10	2.3	5	100	Do.
	13.5	511	78	19	5	10	2.6	5	110	Do.
1928.....	10.2	935	90	21	4	11	2.20	5	140	
	12.4	985	90	23	6.5	11	2.34	7	110	Do.
	12.4	825	85	22	6	11	2.39	5	160	
	12.8	985	95	20	4	11	2.20	7	133	
	12.9	907	90	26	6	11	2.15	6	105	
	13.0	825	90	18	3	11	2.13	6	170	Do.
	13.2	795	92	18	3	11	2.08	5	120	Do.
	13.2	768	90	22	5.5	12	2.26	5	105	
	13.5	985	95	22	4	12	2.20	6	166	
	13.5	796	90	20	3	11	2.29	6	110	
	13.8	1,079	92	20	4	11	2.08	6	130	
	14.0	985	92	22	6	10	1.85	6	150	Do.
	14.0	985	93	21	5	11	2.11	6	155	Do.
	14.0	738	90	21	3.5	10	2.00	5	133	
	14.3	825	90	18	3	11	2.16	5.5	210	
	14.6	825	90	20	3	10	1.92	6.5	155	
	14.6	935	97	20	3	11	2.04	7	110	Do.
	14.8	825	88	24	6	12	2.35	5.5	112	
	14.6	907	90	19	4.5	11	2.20	7	112	Do.
	14.7	1,022	92	28	7	11	2.04	7	140	
	14.9	565	78	18	4	10	2.27	5.5	130	Do.
	15.2	765	87	21	5	11	2.08	7	140	
	15.8	710	87	22	5.5	10	2.17	6	103	
	17.8	907	90	18	4	10	2.04	6	160	
	(*)	765	87	18	2.5	12	2.60	6	155	Do.
1612-24:										
1927.....	9.5	738	90	27	7.5	9	2.0	7	153	Do.
	9.9	795	95	22	4	10	2.1	7	130	Do.
	10.2	852	90	21	5	11	2.2	6.5	206	Do.
	10.5	852	93	20	4	11	2.2	7.5	125	Do.
	11.8	795	85	20	4	11	2.5	6	162	Do.
	12.2	738	88	25	7	10	2.3	8	100	Do.
	12.6	852	95	17	3	10	2.1	7	188	Do.
	12.6	1,079	100	20	3.5	12	2.3	7	188	Do.
	12.6	1,079	95	17	4	12	2.3	7	124	Do.
	12.8	1,134	95	23	4	10	1.9	7	90	Do.
	13.0	680	78	24	7.5	9	2.3	6.5	188	Do.
	13.0	625	90	20	5	10	2.0	7	141	Do.
	13.0	795	85	20	5	9	2.0	7.5	206	Do.
	13.2	1,079	95	20	6	11	2.3	7	150	Do.
	13.2	958	98	17	3	13	2.6	6	118	Do.
	13.5	680	85	19	5	11	2.4	5.5	100	Do.
	13.8	738	80	17	3	12	2.8	5	112	Do.
	14.0	738	83	17	2.5	11	2.4	5	135	Do.
	14.3	625	75	20	3	9	2.3	7	159	Do.
	14.4	625	83	22	8	10	2.3	4	141	Do.

* Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
1612-24—Contd. 1928.....	Per cent	Grams	Mm.	Mm.	Mm.	Number		Mm.	Number	
15.0	1,022	100	18	3	11	2.1	-----	190	Absent.	
15.0	825	85	23	3.5	10	1.7	-----	120		
16.2	907	95	23	3	9	1.9	-----	160		
16.2	882	90	23	4	10	2.0	-----	130	Present.	
17.0	937	95	18	2.5	10	2.1	-----	140		
17.0	907	87	21	4	10	2.2	-----	150	Do.	
17.2	1,079	99	22	5	12	2.4	-----	110		
17.2	1,079	93	23	3	11	2.2	-----	150		
17.4	680	87	18	2	12	2.4	-----	160		
17.6	882	100	16	2	11	2.1	-----	110		
17.7	738	83	18	4	12	2.7	-----	140		
18.0	882	90	14	2	11	2.2	-----	160		
18.2	710	80	13	2	11	2.5	-----	190	Absent.	
18.3	882	89	16	3	11	2.1	-----	160	Do.	
18.6	907	88	22	6.5	11	2.2	-----	150	Do.	
18.6	680	78	18	5	10	2.4	-----	160		
18.6	710	87	16	2	10	2.1	-----	150		
19.0	1,134	104	18	3.5	13	2.3	-----	170	Do.	
19.6	852	95	19	4	11	2.2	-----	210		
20.0	738	90	15	2	11	2.2	-----	150		
(*)	625	90	18	3	11	2.3	-----			
(*)	958	97	20	3	10	2.0	-----			
(*)	882	90	15	2	12	2.3	-----			
(*)	710	93	15	2.5	12	2.3	-----			
(*)	710	90	20	3	10	2.2	-----			
18458-25: 1927.....										
11.2	1,361	110	19	3	10	1.8	7	131	Do.	
12.5	1,134	95	20	3	10	2.0	8	144	Do.	
12.6	795	88	21	3	10	2.2	7	100	Do.	
12.9	738	80	20	3	10	2.6	8	137	Do.	
12.9	852	84	19	5.5	10	2.3	7	106	Do.	
13.5	680	83	22	3	10	2.4	5	119	Do.	
13.5	958	93	20	4	10	2.0	8	119	Do.	
13.6	680	80	17	3	10	2.4	6	87	Do.	
13.6	958	100	24	3.5	11	2.3	7	100	Do.	
14.0	738	82	19	4	11	2.6	6	113	Do.	
14.1	907	95	17	2	10	2.0	5.5	106	Do.	
14.1	852	90	19	3	11	2.2	7	125	Do.	
14.3	680	82	18	3	9	2.2	6.5	113	Do.	
14.5	795	93	20	3	10	2.1	7	131	Do.	
14.6	738	90	19	2	10	2.0	6	94	Do.	
14.8	738	84	19	3	9	2.1	8	144	Do.	
14.8	625	73	12	1	13	3.4	5	100	Do.	
15.0	852	85	22	4	9	2.2	8	193	Do.	
15.0	852	85	11	1.5	12	2.6	5.5	119	Do.	
15.8	852	95	13	3	14	2.9	7	144	Do.	
2287-24 1927.....										
11.4	1,134	105	30	9	10	2.0	7	161	Do.	
11.6	795	80	27	6.5	8	2.0	8.5	195	Do.	
12.0	738	85	23	6	10	2.3	7	150	Present.	
12.0	738	85	26	6	8	1.9	7	172	Absent.	
12.2	738	80	20	5	9	2.2	6	140	Do.	
12.5	852	93	28	6	9	2.0	8	131	Present.	
12.7	958	90	26	5	9	2.0	6	150	Absent.	
12.8	852	90	30	10	9	2.1	5.5	121	Do.	
12.8	680	80	22	5	9		7	192	Do.	
12.8	738	85	30	7	8	2.0	7	121	Do.	
13.0	958	105	26	5	10	1.8	4	142	Do.	
13.2	625	80	20	5	10	2.4				
13.3	907	88	33	14	9	1.8	7	136	Do.	
13.3	738	80	28	6	8	1.9	7	164	Do.	
13.4	625	75	17	2	11	2.8	5	163	Do.	
13.6	958	95	26	8	10	2.1	8	138	Do.	
13.8	1,134	95	32	6	9	2.1	6	142	Do.	
14.0	795	90	22	5	12	2.6	7	182	Do.	
14.0	1,022	95	30	8	10	1.9	6	134	Do.	
14.0	625	80	27	6	8	1.9	6	166	Do.	
14.1	738	90	24	5	9	2.0	5	158	Do.	
14.6	1,022	90	23	5	11	2.2	6	191	Present.	
14.6	625	80	22	5	9	2.2	6	138	Do.	
14.7	795	85	27	6	9	2.0	4	182	Absent.	
14.9	795	83	22	5	9	2.1	7	142	Do.	

* Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vas- cular zone	Vascu- lar bun- dles per decim- eter	Lignified sheath cells
2310s-25: 1927-----	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
	11.1	1,134	98	33	12	10	2.1	6	192	Present.
	12.1	907	100	22	3.5	14	3.0	6	141	Absent.
	12.3	1,361	115	24	5	13	2.4	5	183	Do.
	12.4	1,022	98	22	7	10	2.0	7	190	Do.
	12.5	625	88	20	5	10	2.2	4	120	Do.
	12.6	1,079	100	28	7	10	2.0	7	179	Do.
	13.0	1,079	100	20	3.5	8	2.1	7	170	Do.
	13.0	1,306	110	28	4	11	1.9	9	160	Do.
	13.1	907	85	20	4	12	2.6	6	143	Present.
	13.2	937	90	15	4	9	2.0	6	216	Do.
	13.3	907	100	23	6	10	2.2	7	123	Absent.
	13.3	625	83	24	8	8	2.2	6	200	Do.
	13.4	734	95	22	6	11	2.3	6	223	Do.
	13.5	738	93	20	6	10	2.2	5.5	141	Do.
	13.6	907	100	23	6	10	2.2	4.5	150	Do.
	13.8	738	95	19	3	10	2.3	5	193	Present.
	13.8	738	90	20	5	11	2.5	6	120	Absent.
	14.0	1,134	115	22	5	12	1.7	6	115	Do.
	14.1	625	88	18	4	9	2.5	7	157	Do.
	14.1	907	95	22	5	11	2.3	7	149	Do.
	14.1	1,022	100	27	7	13	2.6	4	143	Do.
	14.4	1,022	100	19	5	13	2.7	6	158	Do.
	14.5	907	95	21	3.5	10	2.2	7	190	Do.
	14.8	1,079	103	24	7	11	2.2	6	161	Do.
	15.0	958	98	15	3	13	2.5	7	140	Do.
2445s-25: 1927-----										
	12.5	1,022	98	17	3	10	2.2	8	200	Present.
	12.7	917	95	24	5	10	1.7	8	200	Do.
	12.7	568	75	25	7.5	9	2.4	7	157	Absent.
	12.9	680	75	22	3.5	9	2.3	6	213	Present.
	13.1	907	93	26	4	11	2.5	7	177	Do.
	13.2	680	73	18	2	9	2.4	5	210	Do.
	13.2	680	78	19	5	10	2.4	7	200	Do.
	13.3	1,420	100	21	3.5	12	2.3	7	177	Do.
	13.8	341	90	21	3	12	2.5	5	210	Do.
	13.8	625	75	25	5	9	2.1	6	154	Do.
	13.8	907	85	18	2	9	1.9	7	110	Absent.
	14.1	568	73	20	3.5	9	2.6	7	200	Do.
	14.3	680	90	27	6.5	11	3.3	8	193	Present.
	14.3	958	85	30	8	8	2.0	7	228	Do.
	14.4	738	85	21	3.5	11	2.4	6.5	220	Absent.
	14.7	738	90	16	2	10	2.6	6.5	218	Do.
	14.7	852	90	18	2	10	2.3	7	110	Do.
	14.8	680	80	25	4.5	11	2.7	7	166	Present.
	15.0	738	78	17	3	12	2.8	6	163	Do.
	15.1	568	70	23	4.5	11	2.1	6	200	Do.
	15.1	1,079	90	16	2	11	2.3	6	169	Do.
	15.1	738	80	18	2	11	2.7	5	210	Do.
	15.6	568	70	22	3.5	9	2.6	5	166	Do.
	15.8	1,022	90	30	4.5	11	2.7	8	191	Do.
	15.8	738	80	16	2.5	14	3.4	4.5	150	Absent.
3403-24: 1927-----										
	8.3	962	88	20	4	10	2.3	5.5	133	Do.
	8.5	1,361	108	23	7	12	2.3	6	123	Present.
	9.0	1,250	90	22	5	10	2.1	6	142	Absent.
	9.5	1,193	95	19	4	12	2.4	4	133	Do.
	9.8	738	75	26	9	9	2.1	5	150	Do.
	9.8	1,306	100	27	9	11	2.4	6	150	Present.
	9.9	958	85	22	6	10	2.1	5	142	Absent.
	9.9	1,420	110	29	5	9	1.7	6	175	Do.
	9.9	958	90	13	3	9	2.4	4	190	Present.
	10.0	958	103	24	8	4	9	2.1	150	Present.
	10.0	1,590	80	20	5	11	1.8	9.5	143	Present.
	10.2	1,134	80	20	5	11	2.6	6	210	Absent.
	10.5	1,079	100	29	9	10	2.1	6	116	Do.
	10.8	1,022	90	26	7	9	1.8	5	158	Do.
	11.0	1,558	90	22	8	10	2.2	4	139	Do.
	11.0	1,134	85	22	4	11	2.3	5	150	Do.
	11.0	738	80	22	4	8	2.0	6	131	Do.
	11.3	1,022	90	29	9	9	2.2	5	100	Do.
	11.7	738	80	20	5	11	2.7	5	150	Present.
	11.9	958	90	19	4	10	2.4	6	130	Do.

TABLE 2.—Macroscopic, chemical, and microscopic data on mature bees grown at Fort Collins, Colo., in 1927 and 1928—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coeff- cient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
3403-24—Contd	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
1927-----	12.1	680	80	23	6	9	2.3	5	140	Absent.
	13.0	852	80	23	7	8	2.0	5	180	Do.
	13.3	1,134	88	20	6	11	2.6	6	166	Do.
	13.6	852	85	20	3	9	2.3	7	210	Present.
	14.1	907	83	18	3	10	2.3	6	190	Absent.
1928-----	14.0	1,361	98	20	3.5	11	2.0	-----	90	Do.
	14.8	1,022	95	23	5	11	2.1	-----	140	
	16.4	825	75	17	2.5	10	2.1	-----	100	Present.
	16.8	825	80	17	4	9	2.2	-----	130	
	17.0	852	80	18	4	11	2.2	-----	90	Absent.
	17.0	1,022	94	19	4	12	2.3	-----	160	Present.
	17.0	907	82	20	3	11	2.4	-----	120	Do.
	17.2	655	70	18	4	9	2.3	-----	110	Absent.
	18.8	958	85	17	2.5	11	2.4	-----	130	Present.
	18.0	795	77	16	2.5	11	2.7	-----	130	
	18.0	795	78	19	4	9	2.1	-----	150	
	18.6	907	80	20	4	10	2.1	-----	150	Absent.
	20.0	541	70	12	1.5	11	2.7	-----	150	
	20.0	882	85	14	1.5	11	2.5	-----	140	
	20.2	710	77	10	1.5	12	2.7	-----	140	Do.
	(a)	1,079	90	22	6	10	1.9	-----	110	Do.
	(a)	655	76	19	5	11	2.5	-----	120	
	(a)	882	91	21	6	11	2.2	-----	120	
	(a)	680	75	18	4	9	2.2	-----	110	
	(a)	680	82	20	4	10	2.3	-----	120	
	(a)	511	74	11	1	12	2.6	-----	-----	
	(a)	568	72	12	2	13	3.0	-----	-----	
	(a)	483	67	12	1.5	11	3.2	-----	-----	
	(a)	795	76	14	1.5	11	2.4	-----	-----	
	(a)	453	67	14	2	10	3.0	-----	-----	
3956-24:										
1927-----	11.2	1,134	100	20	3.5	10	2.1	5	142	Do.
	11.5	1,306	103	20	4	10	2.2	5	110	Do.
	11.7	1,079	105	23	7	11	2.1	5	142	Do.
	11.8	680	80	20	5.5	10	2.5	6	125	Do.
	12.0	1,134	95	20	5	11	2.3	7	117	Do.
	12.2	1,818	115	25	7	12	2.0	7	110	Do.
	12.4	1,306	117	15	5	14	2.6	7	98	Do.
	12.5	852	88	19	4	15	3.0	6	105	Do.
	12.5	795	95	21	5	10	2.0	6	110	Do.
	12.6	1,306	108	21	4	14	2.5	6	117	Do.
	12.7	852	95	17	4	12	2.5	5	100	Do.
	12.8	958	100	15	2.5	11	2.1	6	177	Do.
	13.0	1,079	95	21	5	12	2.5	6	98	Do.
	13.2	1,134	95	25	7	12	2.3	5	80	Do.
	13.3	852	95	10	1.5	14	2.6	6	120	Do.
	13.4	907	85	16	3	14	3.2	5	102	Do.
	13.4	907	90	20	4	10	2.1	8	142	Do.
	13.5	1,134	103	19	4	12	2.4	6	148	Do.
	13.8	852	90	18	4	12	2.3	4	145	Do.
	14.0	907	95	13	3	12	2.6	4	148	Do.
	14.1	1,022	108	18	5	14	2.7	7	180	Do.
	14.6	958	93	17	5	14	2.7	6	132	Do.
	15.0	958	100	18	4	14	2.7	8	125	Do.
	15.8	795	90	15	3	14	3.0	5	140	Do.
	16.4	738	85	16	3	15	3.1	4	127	Do.
1928-----	15.2	710	90	9	4	17	3.15	5	100	
	15.8	1,022	105	23	5.5	16	2.71	7	71	
	16.2	795	90	21	6	13	2.55	5	134	
	16.3	958	100	15	2	14	2.33	7	108	
	16.6	680	87	18	3	12	2.50	6.5	72	
	16.8	907	102	18	3	12	2.18	7	94	
	17.0	907	95	17	2.5	16	3.02	6	79	
	17.6	1,022	105	18	3.5	14	2.41	7	66	
	17.6	795	95	15	2.5	13	2.45	6	107	
	18.0	907	94	16	2.5	11	2.08	7	132	
	18.0	680	87	17	3.5	14	3.04	6	80	
	18.0	680	90	13	3	14	2.45	6	100	
	18.0	795	87	17	3	13	2.76	6	89	
	18.2	795	95	15	2.5	15	2.83	6	104	
	18.2	738	90	12	2	16	3.13	6.5	116	
	18.4	680	87	13	2	14	2.58	5	130	

• Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
3956-24—Contd. 1928	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
	18.8	852	98	17	3.5	13	2.45	6.5	65	
	18.8	907	97	18	4	14	2.59	6	104	
	19.0	1,100	107	16	2.5	17	2.83	6	81	
	19.2	680	85	12	2.5	17	3.69	5	70	
	(*)	1,022	102	15	2.5	13	2.38	6.5	99	
	(*)	540	83	17	3.5	14	3.04	6	86	
	(*)	795	94	16	3	15	2.68	7	108	
	(*)	852	98	18	3	14	2.54	8	129	
	(*)	680	85	14	3.5	14	2.99	5	94	
4545-24: 1927										
	10.1	958	85	14	2.5	13	2.6	7	183	Absent.
	11.1	568	73	22	5.5	11	2.8	4	145	Do.
	12.1	680	78	25	7.5	9	2.2	5	182	Present.
	12.3	453	68	23	6	10	2.8	5	166	Do.
	12.7	1,079	85	23	6	11	2.5	5	-----	Absent.
	13.1	1,022	83	26	6	9	2.1	6	200	Present.
	13.2	680	80	18	4.5	11	2.8	5	190	Do.
	13.2	511	65	17	4.5	12	3.2	5	136	Do.
	13.3	511	68	22	6	10	2.9	6	210	Do.
	13.4	795	80	22	4.5	9	2.1	7	150	Absent.
	13.5	1,079	88	21	3	10	2.3	6	180	Do.
	13.5	795	83	22	3.5	11	2.6	7	210	Do.
	13.7	625	75	24	9	12	3.1	4	166	Do.
	13.8	1,079	90	24	9	11	2.1	6	-----	Do.
	13.9	1,022	83	21	5.5	10	2.5	5	175	Do.
	14.1	568	68	-----	6.5	10	2.6	4	200	Present.
	14.3	580	73	16	2.5	11	3.0	4	173	Do.
	14.5	738	78	18	3	11	2.6	6	137	Do.
	14.7	738	75	19	3.5	12	2.8	5	245	Do.
	14.8	738	73	20	3	12	3.0	4	190	Do.
	14.8	852	90	19	5	15	3.2	4	120	Absent.
	15.3	852	73	20	3.5	12	2.9	4	166	Do.
	15.3	511	65	17	3.5	10	2.8	5	177	Do.
	16.0	568	65	22	5.5	12	3.4	3	144	Do.
	16.6	625	75	18	3	11	2.9	5	180	Do.
1928										
	15.2	655	70	18	2	13	3.1	-----	150	Present.
	15.4	907	85	25	3.5	11	2.5	-----	140	Do.
	15.4	655	72	23	6	10	2.4	-----	160	Do.
	15.4	1,022	77	17	2.5	10	2.3	-----	136	Do.
	15.8	680	70	18	4	10	2.3	-----	120	Absent.
	16.2	655	75	22	5	11	2.5	-----	140	Present.
	16.3	568	73	18	2.5	10	2.4	-----	130	Do.
	16.7	1,134	87	24	5.5	12	2.3	-----	120	Absent.
	16.9	935	80	22	3	14	2.8	-----	140	Present.
	17.0	825	75	23	5	11	2.4	-----	130	Do.
	17.0	880	80	17	2	12	2.4	-----	140	Do.
	17.5	710	70	14	2	12	2.8	-----	130	Do.
	17.6	907	87	21	4	15	3.0	-----	150	Do.
	17.6	598	72	22	3	10	2.4	-----	170	Do.
	18.0	680	68	20	5	13	3.2	-----	120	Do.
	18.0	880	72	22	4	12	2.6	-----	150	Absent.
	18.2	511	70	18	2.5	11	2.9	-----	180	Present.
	18.8	680	73	16	3	13	2.6	-----	110	Do.
	19.0	765	74	17	2.5	11	2.6	-----	170	Do.
	19.0	540	63	20	3	10	2.8	-----	140	Do.
	(*)	935	80	22	3	11	2.4	-----	140	-----
	(*)	625	63	16	5	12	2.6	-----	130	-----
	(*)	540	68	23	5	10	2.4	-----	-----	-----
	(*)	511	68	22	7	10	2.6	-----	-----	-----
	(*)	795	72	16	3	12	2.6	-----	-----	-----
5656-24: 1927										
	10.1	1,022	95	26	4	11	2.2	6	183	Absent.
	11.4	958	90	22	4.5	10	2.1	6	154	Do.
	11.4	1,134	100	30	9	9	1.7	7	169	Do.
	11.7	1,022	100	30	6	9	1.8	6	170	Do.
	12.0	795	85	22	4	10	2.2	6	170	-----
	12.2	1,079	90	21	5	11	2.1	6	160	-----
	12.2	795	93	25	6	11	2.1	7	240	-----
	12.2	795	93	23	6	11	2.3	8	166	-----
	12.4	1,134	100	25	4.5	9	1.8	5.5	170	-----
	12.6	680	75	20	3	9	2.3	4	158	-----
	12.6	907	88	25	5	8	1.8	8	183	-----

* Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
5556-24—Contd.	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
1927.....										
	12.6	1,079	85	23	6	10	2.3		7	230
	12.6	852	93	23	5	8	1.8		6	200
	12.7	907	88	18	4	12	2.6		7	205
	12.8	907	90	23	6	10	2.1		7	276
	12.9	568	85	22	4.5	11	2.7		4	175
	13.0	795	85	23	7.5	10	2.2		5	175
	13.0	738	75	18	3	9	2.2		4	190
	13.1	958	93	24	5	10	2.1		8	180
	13.3	1,022	105	25	4	10	2.0		5	211
	13.5	680	78	22	6.5	10	2.1		4	189
	13.5	625	73	17	4	10	2.8		6	142
	14.4	795	83	23	5	8	2.0		6	158
	14.6	738	83	20	4.5	11	2.5		6	182
	14.9	511	75	20	3	11	2.7		5	210
1928.....										
	13.2	568	80	24	7	10	2.1		6	140
	14.3	795	90	20	4	10	2.0		7	157
	15.2	1,164	95	26	7	10	1.9		7	116
	15.4	1,134	100	22	4.5	9	1.6		8	160
	15.6	937	98	27	6.5	9	1.8		7	167
	16.2	795	83	24	4.5	9	2.2	6.5	110	
	16.2	937	93	22	4.5	11	2.0		6	113
	16.6	540	77	20	5	9	2.0		5	130
	16.6	852	90	18	5	11	2.1		6	103
	17.0	1,022	82	23	5	10	1.9		6	116
	17.0	710	70	19	4	10	2.3		4	105
	17.0	568	75	23	7	10	2.3		5	130
	17.4	882	86	24	5.5	10	2.1		6	140
	17.5	768	92	22	4	11	2.2		6	150
	17.8	710	95	25	6	10	2.2		7	116
	18.2	825	90	20	4	12	2.2		5	98
	18.2	568	73	24	7	9	2.1		6	193
	18.3	710	77	22	4	10	2.1		7	133
	19.0	1,134	97	18	3	11	1.9		5	116
	19.9	598	77	18	3.5	10	2.4		6	175
	(*)	312	65	14	3	10	2.7		5	143
	(*)	568	75	16	2.5	12	2.6		4	123
	(*)	483	82	18	3	10	2.2		5	153
	(*)	453	70	18	4	9	2.1		5	123
	(*)	483	67	15	4	12	3.0		5	127
5942-24:										
1927.....										
	11.3	1,022	95	25	7.5	11	2.1		7	200
	11.3	680	85	27	7	9	2.1		8	80
	11.6	680	75	21	4.5	11	3.2		7	160
	12.0	1,022	90	23	7	10	2.3		7	180
	12.4	680	80	23	4.5	11	2.6		7	75
	12.4	680	80	22	4.5	9	2.3		7	180
	12.6	625	80	20	4	10	2.6		6	123
	12.7	1,250	95	25	5.5	13	2.5		8	150
	13.1	1,250	105	24	4.5	11	2.0		7	143
	13.2	958	90	25	5.5	9	2.1		6	193
	13.3	738	85	22	4.5	11	2.4		6	170
	13.4	1,590	105	27	5	11	2.1		8	100
	13.6	738	85	26	5	10	2.5		7	159
	13.6	1,022	90	25	6	11	2.4		6	125
	13.6	568	80	21	3.5	10	2.4		8	115
	13.8	852	95	23	5	11	2.3		7	145
	13.8	853	93	22	4	11	2.4		7	200
	14.1	625	80	20	3.5	11	2.7		7	121
	14.9	1,134	100	22	8.5	11	2.3		8	140
	15.0	568	80	18	3.5	11	2.8		4	108
	15.0	795	90	25	3.5	10	2.1		7	140
	15.1	795	95	23	5	13	2.9		5	110
	15.1	907	95	16					5	
	15.2	907	80	18	4.5	10	2.5		4	130
	15.8	625	83	20	5	11	2.6		6	120
1928.....										
	15.1	680	85	15	3.5	11	2.29		7	94
	15.4	1,052	100	27	8	10	1.82		7	83
	15.6	738	85	19	5	11	2.24		6	140
	16.5	1,134	105	21	6	12	1.90		8	140
	16.6	625	80	20	4	10	2.04		6	110
	16.6	568	76	15	2	13	2.32		7	99

* Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued*

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coeffi- cient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
5942-24—Contd. 1928-----	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
	16.6	710	80	20	3.5	10	2.17	5.5	120	Absent.
	16.8	795	92	18	3	11	2.20	5	120	
	17.0	680	84	16	3	12	2.50	4.5	200	
	17.4	795	85	20	3.5	12	2.40	4	130	Do.
	17.4	652	82	22	5.5	10	2.30	5.5	122	
	17.5	765	85	17	1.5	12	2.45	5	83	
	17.8	907	93	23	5.5	11	2.10	8	150	Do.
	17.9	820	90	20	3	13	2.29	6	99	
	18.0	588	80	21	2.5	11	2.75	5	84	
	18.2	985	100	17	3	12	2.32	8	100	Do.
	18.4	795	87	18	2.5	12	2.40	5	160	
	18.5	765	85	20	4.5	12	2.53	5.5	90	
	18.6	680	82	17	4	11	2.20	5.5	170	Do.
	18.6	1,022	95	19	3	13	2.24	5	106	
	18.7	1,022	97	22	3.5	11	2.10	8	190	
	19.0	907	98	13	1.5	12	2.18	6	100	
	19.0	540	76	14	2	13	2.60	6	105	
	19.0	680	82	15	3	13	2.95	5	72	
	19.8	595	78	22	6	11	2.20	4	72	
7794-24: 1927-----										
	8.2	738	88	27	8	9	2.0	6	161	Present.
	8.6	907	95	30	7	11	2.3	6	172	Absent.
	8.9	738	80	19	4	10	2.5	5	120	Do.
	9.1	907	98	24	5	10	2.0	6.5	166	Present.
	9.8	738	93	22	4.5	11	2.3	6	90	Absent.
	10.1	958	90	21	8	9	2.1	6	175	Do.
	10.2	1,134	110	22	6	12	2.1	6	227	Present.
	10.2	680	78	18	3	11	2.8	3	200	Absent.
	10.4	852	85	16	4	12	2.2	4	154	Present.
	10.6	738	83	18	3	12	2.7	4	127	Do.
	10.7	1,022	85	24	3	11	2.1	6	160	Do.
	11.1	738	78	20	6	13	3.3	4	136	Absent.
	11.2	1,420	108	29	8.5	12	2.1	8	163	Present.
	11.9	738	83	25	5	8	2.0	6	208	Do.
	12.0	1,022	95	21	8	12	2.3	6	145	Do.
	12.0	738	90	22	4	10	2.2	5	166	Do.
	12.3	958	88	22	5	10	2.2	5	166	Do.
	12.3	738	78	21	3.5	8	2.0	5	200	Absent.
	12.4	1,022	85	22	3	9	2.1	4	168	Present.
	12.7	958	95	23	5	12	2.3	5	200	Do.
	12.8	1,280	108	30	11	11	2.0	7	154	Absent.
	12.9	625	74	21	5	11	3.0	6	122	Do.
	13.2	852	85	28	9	12	3.0	7	140	Do.
	13.2	958	73	22	4	10	2.4	5	172	Do.
	13.3	568	73	18	4	15	3.5	4	200	Present.
1928-----										
	13.2	795	85	15	2	12	2.26	6.5	144	
	14.2	738	84	18	2.5	9	2.25	6.5	123	
	14.2	738	92	18	3	10	2.09	8	113	
	14.4	795	95	20	4	10	2.13	7	144	
	14.4	650	85	17	2	10	2.44	4	143	
	14.6	710	83	21	4.5	11	2.75	6	146	
	14.7	1,050	88	21	5	13	2.32	6.5	147	
	15.0	907	85	20	5	11	2.29	6	143	
	15.0	765	80	20	3.5	11	2.44	5.5	130	
	16.0	825	92	17	2.5	10	2.00	7	142	
	16.0	765	86	17	4	11	2.24	7	132	
	16.2	907	90	20	4	13	2.45	7	143	
	16.4	907	75	18	2.5	11	2.55	5	149	
	16.5	1,022	95	22	6	11	2.20	6.5	92	
	16.6	907	95	17	2	9	1.76	6.5	140	
	16.7	650	75	17	3	11	2.62	5	130	
	17.2	1,022	100	18	3.5	12	2.03	6	134	
	17.3	935	86	22	4	12	2.40	6	131	
	17.3	480	78	19	3.5	10	2.27	4	138	
	17.6	680	83	20	3	10	2.32	6	110	
	17.8	625	75	18	4	11	2.55	4	92	
	18.6	568	68	15	2.5	11	2.44	4.5	145	Absent.
	18.8	907	82	21	6	12	2.29	5	94	
	19.5	595	74	17	3	13	2.88	5	107	

TABLE 2.—Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
8753-24: 1927-----	<i>Per cent</i>	<i>Grams</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Number</i>		<i>Mm.</i>	<i>Number</i>	
	11.3	680	88	24	5	10	2.2	6	125	Absent.
	12.1	680	98	26	5	9	1.7	8	191	Do.
	12.1	1,134	98	25	5.5	9	1.9	8	163	Do.
	12.7	625	75	26	7	9	2.4	5	146	Present.
	12.7	1,306	103	21	3	8	1.5	7	138	Absent.
	12.9	1,134	105	30	8.5	10	2.0	6.5	123	Do.
	13.3	852	85	20				5.5	140	Do.
	13.5	1,022	100	20	4	13	2.3	6	164	Do.
	13.6	852		23	4	10	2.1	6.5	177	Present.
	13.6	795	88	25	6	8	1.7	6.5	161	Do.
	13.7	1,079	90	28	6	8	1.8	8	166	Do.
	13.9	795	93	21	6	10	2.0	6.5	170	Absent.
	14.0	852	92	25				7	148	Do.
	14.1	852	93	23	5.5	10	2.0	7	100	Present.
	14.2	795	88	25	5.5	9	2.0	5	142	Absent.
	14.3	1,022	90	20	6	8	1.6	6	158	Do.
	14.3	738	80	27	11	9	2.3	6	142	Do.
	14.7	680	80	26	8	8	2.0	6.5	177	Do.
	14.7	680	90	25	4	8	2.0	4	125	Do.
	14.8	907	85	20	4	11	2.5	5	100	Present.
	15.0	628	80	22	4	9	2.0	7	175	Absent.
	15.1	680	83	16	4	9	2.3	4	138	Present.
	15.2	907	90	22	5	9	2.1	6	171	Do.
	15.2	1,134	103	19				7	160	Absent.
	15.2	738	88	20	5	11	2.6	4	191	Present.
1928-----	15.2	852	93	22	5	11	2.2		120	Absent.
	15.3	1,052	100	18	4	12	2.2		130	Do.
	15.4	680	85	25	4	9	1.9		160	
	15.8	795	92	21	5	10	1.9		160	
	16.2	953	99	30	9	10	1.9		80	Absent.
	16.2	907	95	25	5	10	2.0		130	
	16.4	1,022	95	25	5.5	11	2.2		130	Absent.
	16.6	738	81	18	5	9	2.0		230	
	16.8	988	100	23	5.5	10	1.8		120	
	17.0	825	95	17	3	10	2.0		140	Do.
	17.0	795	87	17	3	11	2.3		170	
	17.4	768	88	21	3	11	2.3		100	Do.
	17.6	710	92	22	5	9	1.8		130	
	17.6	1,022	90	15	3	10	2.1		130	
	17.7	795	89	24	5	11	2.0		110	
	18.0	768	81	20	4	12	2.8		90	Present.
	18.4	568	84	19	4	9	2.1		90	Absent.
	18.5	795	90	25	4.5	10	1.9		140	Do.
	19.1	768	85	19	4	10	2.1		120	
	19.2	710	87	20	4	10	2.1		120	Present.
	(*)	655	80	17	3	10	2.1			
	(*)	625	75	18	3	9	2.2			
	(*)	768	83	21	4	10	2.3			
	(*)	1,250	105	24	6	11	1.7			
	(*)	1,079	104	23	5	9	1.7			

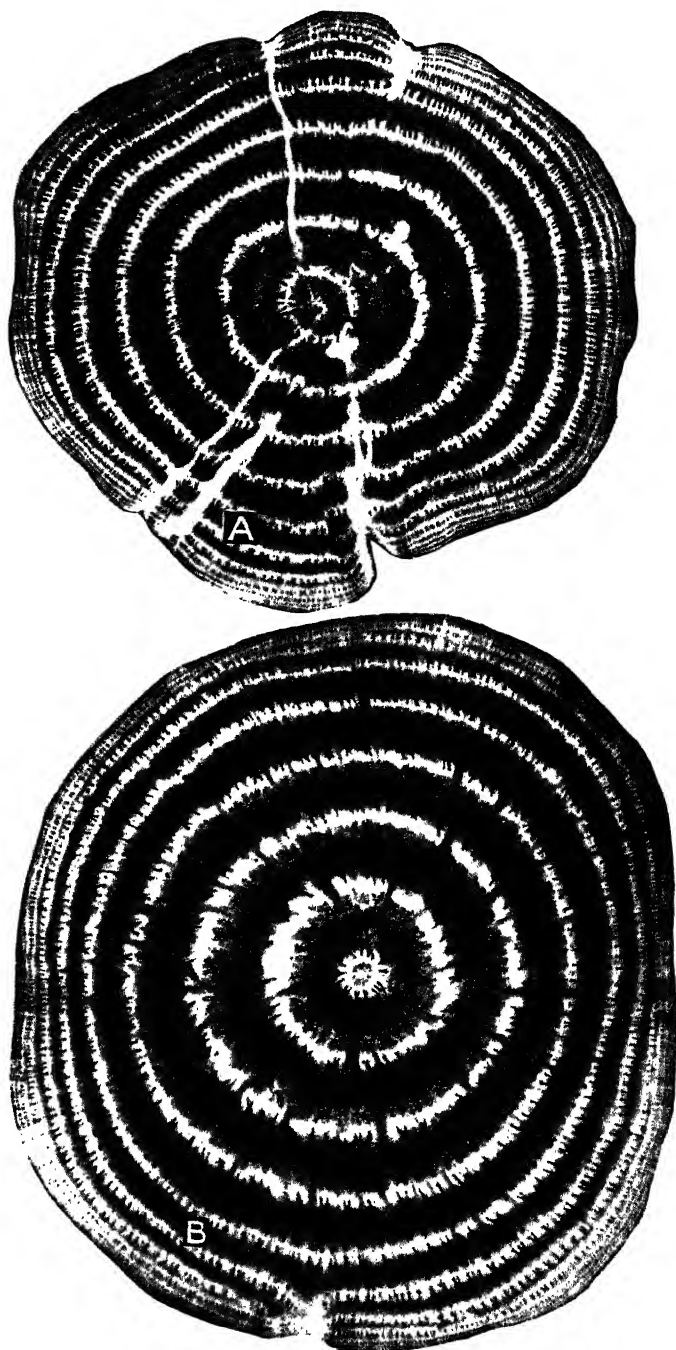
TABLE 3.—*Mean (M), standard deviation (σ), and coefficient of variability (CV) of diameters of first ring and central core, of ring numbers, of ring-density coefficients, and of numbers of bundles per decimeter in the fourth ring of 30 sugar-beet selections*

(Selections listed in numerical order)

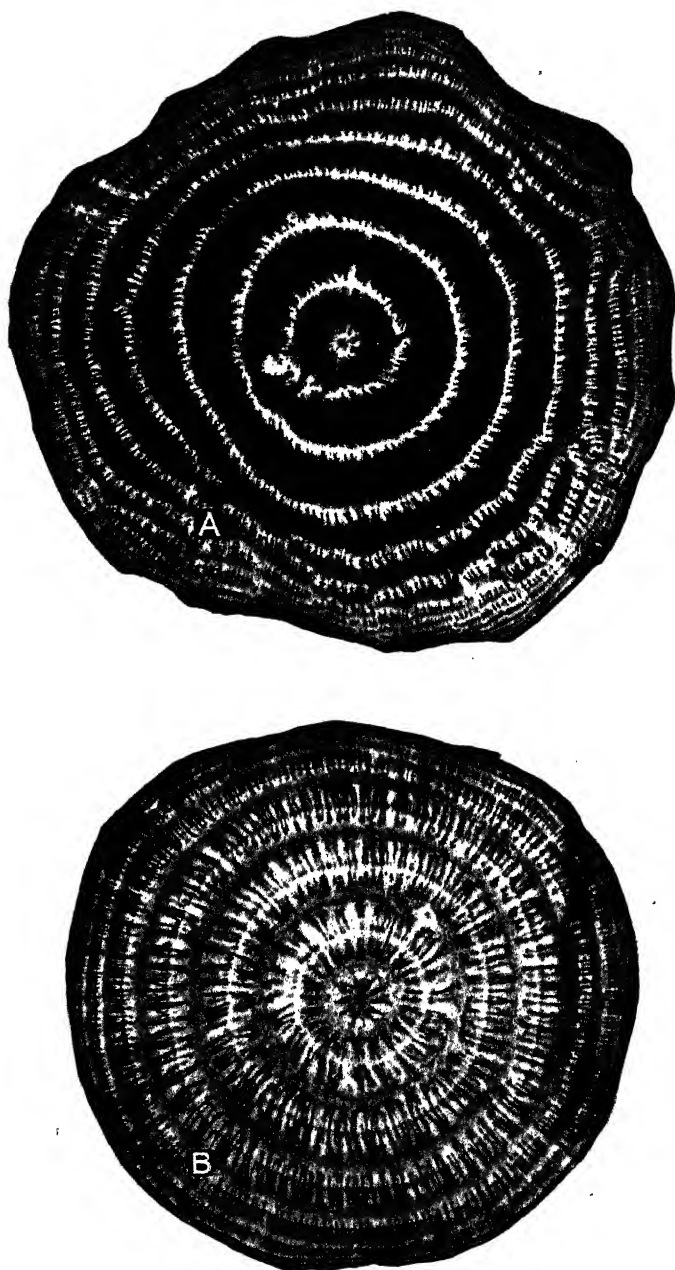
Selection No.	Grown		First ring			Central core			Number of rings			Ring-density coefficient			Number of bundles per decimeter of fourth ring		
	Locality	Year	M	σ	CV	M	σ	CV	M	σ	CV	M	σ	CV	M	σ	CV
112088			<i>M/m.</i>			<i>M/m.</i>											
30																	
130-24	Fort Collins	1927	23.4	3.58	15.30	3.7	1.69	29.65	10.7	1.27	12.70	2.25	0.247	10.98	156	28.3	18.14
	do.	1928	20	2.86	14.30	3.6	1.01	28.13	10.7	1.22	11.40	2.18	.217	9.95	156	24.0	15.38
	Fort Lewis	1928	18.3	3.23	19.60	4.1	1.82	46.93	10.4	1.03	10.30	2.00	.579	28.95	139	29.3	21.08
103R-26	Fort Collins	1927	17.5	3.43	19.60	3.5	1.13	40.00	9.7	1.11	10.67	2.25	.256	11.48	166	41.9	25.24
554-24	do.	1927	18	3.37	18.72	3.5	1.13	37.67	9.7	1.08	11.13	2.25	.373	16.58	174	28.8	16.55
	do.	1928	15	2.31	15.40	2.5	1.04	31.20	10.8	1.27	12.68	2.48	.269	10.85	165	15.0	9.99
	Fort Lewis	1928	15	2.37	16.89	2.5	1.04	31.20	10.8	1.27	12.68	2.50	.321	12.84	162	13.1	21.78
6006-25	Fort Collins	1927	22	3.70	16.89	4.6	2.07	39.00	10.4	1.44	13.85	2.19	.244	11.14	138	25.4	18.40
	do.	1928	19	3.73	19.95	4.3	1.49	32.39	11.2	1.43	12.77	2.04	.227	11.13	137	27.3	19.93
	Fort Lewis	1928	18	3.83	20.16	3.3	1.08	22.79	9.9	1.24	12.52	2.04	.211	10.34	119	16.0	13.44
730-24	Fort Collins	1927	17	3.53	20.76	3.5	1.01	28.86	10.1	1.00	9.90	2.38	.328	13.78	149	23.3	15.64
	do.	1928	17	3.47	16.06	5.5	2.10	38.18	10.4	.98	9.42	2.12	.226	10.66	164	28.8	18.70
808-24	Fort Collins	1927	21.6	3.47	13.32	4.4	1.33	30.23	10.6	1.17	11.58	2.06	.271	13.16	147	40.8	27.75
	do.	1928	19.6	2.61	13.32	4.6	1.18	25.65	11	.92	8.85	2.26	.199	8.80	186	25.4	18.68
	Fort Lewis	1928	17.3	3.17	18.32	4.4	1.33	30.23	10.6	1.77	16.70	2.30	.264	11.48	159	13.6	15.36
9028-25	Fort Collins	1927	20.3	2.97	14.03	4.7	1.44	29.57	12.1	1.26	10.41	2.46	.269	8.50	153	20.1	8.55
	do.	1928	14	3.32	23.71	2.4	1.13	37.92	12.1	.68	11.13	2.13	.353	14.71	166	31.3	13.14
	Fort Lewis	1928	14	3.32	23.71	2.4	1.13	37.92	9.7	1.02	11.13	2.13	.241	11.31	164	29.9	13.42
1161-24	Fort Collins	1927	20	2.43	12.15	4.8	1.47	30.62	9.7	.68	6.39	2.10	.177	8.43	146	17.7	12.12
	do.	1928	15	2.03	13.53	2.6	.60	23.08	9.7	.68	6.39	2.10	.237	11.20	147	19.3	13.13
	Fort Lewis	1928	15	2.48	16.53	3.2	.99	30.94	9.4	.97	10.32	1.94	.191	9.84	149	22.6	13.17
12288-25	Fort Collins	1927	23	2.09	9.09	4.7	1.08	29.14	9.7	.83	8.56	1.96	.191	9.74	120	30.9	26.73
	do.	1928	19	2.62	13.79	3.5	2.86	59.58	10.5	1.12	10.67	2.00	.100	8.09	131	28.3	20.92
	Fort Lewis	1928	19	2.22	11.68	4.3	.96	22.33	10.5	1.12	10.67	2.11	.269	8.67	149	36.8	20.92
12988-25	Fort Collins	1927	19	2.44	14.88	2.9	.83	28.02	10.5	1.38	12.87	2.37	.267	11.27	115	8.8	7.66
	do.	1928	16.4	1.84	14.88	2.9	.96	45.23	10.5	1.38	12.87	2.37	.267	11.27	115	8.8	7.66
	Fort Lewis	1928	11.8	1.96	16.01	2.1	.95	45.23	10.4	.60	9.52	2.26	.199	8.89	155	34.0	21.93
1427-24	Fort Collins	1927	21.6	3.47	16.06	5.5	2.23	40.64	10.4	.60	9.52	2.26	.199	8.89	155	34.0	21.93
	do.	1928	17	3.29	19.35	3.0	1.22	40.67	10.5	1.12	10.77	2.30	.304	12.21	162	33.5	20.08
	Fort Lewis	1928	18	4.57	25.39	3.6	2.40	80.05	10.5	1.30	11.43	2.30	.414	18.00	163	28.5	17.48
1560-24	Fort Collins	1927	22	2.80	12.72	0.0	1.20	26.00	9.7	.62	6.30	2.23	.253	11.35	144	32.8	22.78
	do.	1928	21	2.49	13.86	3.4	1.21	29.32	10.9	.63	5.78	2.17	.155	7.14	136	26.6	19.50
	Fort Lewis	1928	21	2.49	13.86	3.4	1.21	29.32	10.9	.63	5.78	2.17	.155	7.14	136	26.6	19.50
1691-24	Fort Collins	1927	20.4	2.73	13.38	4.7	1.64	34.89	10.5	1.02	9.71	2.26	.213	9.42	140	15.8	16.08
1612-24	do.	1927	20.4	2.73	13.38	4.7	1.64	34.89	10.5	1.02	9.71	2.26	.213	9.42	140	15.8	16.08
	do.	1928	18.5	2.99	16.16	3.2	1.15	35.94	10.9	.89	8.17	2.20	.197	8.95	153	24.7	16.14
	Fort Lewis	1928	19	4.14	21.79	4.3	1.12	26.05	10.9	.89	8.17	2.20	.197	8.95	153	24.7	16.14
													.589	26.77	133	30.6	23.01

TABLE 3.—Mean (M), standard deviation (σ), and coefficient of variability (CV) of diameters of first ring and central core, of ring numbers, of ring-density coefficients, and of numbers of bundles per decimeter in the fourth ring of 30 sugar-beet selections—Continued

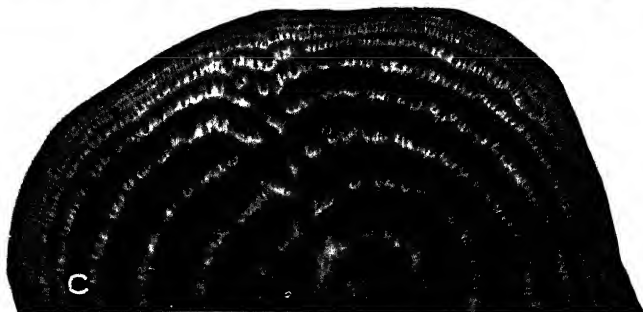
Selection No.	Grown		First ring			Central core			Number of rings			Ring-density coefficient			Number of bundles per decimeter of fourth ring		
	Locality	Year	M	σ	CV	M	σ	CV	M	σ	CV	M	σ	CV	M	σ	CV
1818s-25	Fort Collins	1927	21	3.30	15.71	4.3	1.41	32.79	10.5	1.10	10.47	2.24	.390	17.41	146	30.4	20.82
	do	1928	19	3.12	16.42	2.9	.76	26.21	11.8	.98	8.31	2.27	.279	12.29	155	29.8	19.23
	Fort Lewis	1928	14	4.86	34.70	2.3	1.01	43.91									
1845s-25	Fort Collins	1927	26	3.44	17.84	6.3	2.21	30.32	10.4	1.25	12.02	2.31	.266	11.52	121	23.5	19.42
	do	1927	26	4.23	17.08	6.3	2.21	35.07	9.3	1.01	10.80	2.10	.234	11.14	155	21.7	14.00
	do	1927	22	3.86	17.54	5.4	1.93	35.74	10.8	1.50	14.44	2.28	.274	12.02	162	29.8	18.40
2287-24	do	1927	25	3.81	15.24	5.7	2.12	37.19	11.3	1.01	8.94	2.23	.190	8.52	118	29.4	24.91
2310s-25	do	1927	21	4.17	19.80	3.8	1.65	43.42	10.4	1.33	12.79	2.22	.388	15.52	184	30.9	16.79
	do	1927	21	3.73	16.95	5.8	2.01	34.66	10.7	1.10	11.00	2.40	.242	10.90	162	27.1	17.83
	do	1927	22	3.56	20.94	3.2	1.43	44.69	10.7	1.01	9.44	2.40	.326	13.58	126	19.6	15.55
2446s-25	Fort Lewis	1928	17	3.71	16.13	3.2	1.23	38.43	9.6	1.60	6.25	2.43	.319	13.13	159	17.6	11.07
	do	1928	16	3.42	18.49	4.3	1.35	31.40	12.3	1.64	13.33	2.48	.337	13.59	126	23.8	18.89
	Fort Collins	1927	18.5	2.87	17.94	3.1	.95	30.65	14.2	1.58	11.13	2.70	.553	13.07	97	20.6	21.24
3935-24	do	1928	16	2.44	16.60	2.7	.81	30.00									
	do	1928	14.7	2.44	16.60	2.7	.81	37.14	11	1.34	12.18	2.70	.553	13.07	109	16.4	15.06
	Fort Collins	1927	20	3.06	15.30	4.9	1.82	37.03	11	1.40	12.73	2.60	.249	9.38	140	17.9	12.79
4545-24	do	1928	20	2.97	14.85	3.4	1.90	44.12									
	do	1928	18.5	2.70	14.59	3.4	1.90	44.12									
	Fort Lewis	1927	23	4.49	17.96	6.6	2.44	32.42	10	1.18	11.80	2.23	.312	13.99	101	13.2	7.07
4621-22	Fort Collins	1927	23	3.43	13.61	4.0	1.38	38.72	10.2	1.04	10.40	2.18	.280	13.22	133	23.7	22.33
	do	1928	21	3.39	16.14	4.7	1.38	38.72	10.2	.92	9.02	2.17	.280	13.22	133	23.7	22.33
5609-24	do	1928	19	4.00	13.73	4.1	1.38	38.72	10.6	.88	9.95	2.42	.284	11.73	140	34.4	24.57
	Fort Collins	1927	23	3.00	13.47	5.9	1.38	41.69	11.6	.89	8.53	2.30	.292	10.52	118	34.3	24.07
	do	1928	18	2.72	15.16	4.0	1.13	28.00									
5942-24	Fort Lewis	1927	20	3.95	19.75	3.6	2.29	63.61	9.2	1.04	10.54	2.24	.329	14.60	500	27.9	13.95
	do	1928	15	2.56	17.06	2.0	.78	39.00	10.4	1.26	12.11	2.40	.329	13.58	119	23.4	19.66
	Fort Collins	1928	13	2.63	19.48	1.7	.43	35.29									
7794-24	Fort Lewis	1927	23	3.75	16.30	5.5	2.11	38.36	10.8	1.56	14.44	2.27	.393	17.31	163	33.6	20.61
	do	1928	19	2.00	10.53	3.5	1.16	33.14	10	1.19	11.90	2.38	.377	15.84	164	30.9	18.84
	Fort Collins	1928	19	5.19	27.31	3.8	2.30	60.52									
8146-24	Fort Lewis	1927	21	4.18	19.90	3.7	1.30	35.13	9.6	1.11	11.56	2.32	.361	15.56	154	23.3	15.13
	do	1928	16	2.55	15.36	2.9	.68	23.45	10.7	1.10	10.28	2.15	.298	18.09	144	14.3	9.93
	Fort Collins	1928	15	3.18	21.20	2.3	.77	33.48									
8753-24	Fort Lewis	1927	23	3.07	13.35	5.5	1.78	32.36	9	1.26	14.00	2.04	.277	13.58	152	24.2	15.92
	do	1928	21	3.26	15.52	4.5	2.98	66.22	10	.89	8.95	2.06	.231	11.21	130	32.4	24.92
	Fort Collins	1928	14.5	2.99	20.62	2.4	.82	34.17	9.7	.73	7.52	2.56	.385	15.04	152	29.4	19.34



Cross sections of sugar-beet selections. Natural size: A, No. 4621-22 with 14.6 per cent sugar; B, No. 2346s-25 with 14.2 per cent sugar



Cross sections of beets of selection No. 12938-25. Natural size: A, 16 per cent sugar;
B, 13.1 per cent sugar



Cross section of three sugar beets of selection No. 1845s-25. Natural size: A, 12.5 per cent sugar; B, 15.8 per cent sugar; C, 12.9 per cent sugar

METEOROLOGICAL DATA

The meteorological data for the growing seasons of 1927 and 1928 are given in Table 4. These data show that as compared with the season of 1928 the 1927 season was characterized by a greater rain-fall, slightly higher temperature, and higher relative humidity.

TABLE 4.—*Meteorological data for April to October of 1927 and 1928 at Fort Collins, Colo., with normal data for comparison*

[Furnished by the Colorado Agricultural Experiment Station]

Year and month	Temperature					Relative humidity		Precipitation
	Maximum	Mean maximum	Mean	Minimum	Mean minimum	7 a. m.	7 p. m.	
1927:	° F.	° F.	° F.	° F.	° F.	Per cent	Per cent	Inches
April.....	81.5	58.6	45.2	17.0	31.7	72.2	53.9	2.69
May.....	86.0	71.5	56.3	30.0	41.1	56.0	40.4	.91
June.....	93.0	74.8	61.9	38.9	49.1	76.8	62.9	2.17
July.....	91.0	81.6	67.6	47.6	53.6	70.2	56.0	2.19
August.....	84.8	76.4	64.1	45.0	51.8	81.0	68.4	2.10
September.....	85.2	72.7	58.9	28.0	45.0	73.6	63.4	1.10
October.....	80.5	66.7	50.2	25.0	33.6	75.4	54.6	1.05
Average.....	86.0	71.8	57.7	33.1	43.7	72.2	57.1	-----
Total.....	-----	-----	-----	-----	-----	-----	-----	12.21
1928:	-----	-----	-----	-----	-----	-----	-----	-----
April.....	75.6	57.8	43.3	8.8	28.9	63.0	42.2	.98
May.....	87.1	68.4	55.7	31.1	43.0	70.2	64.9	3.35
June.....	83.0	68.9	57.3	35.8	45.7	75.8	62.8	2.73
July.....	91.0	82.2	67.9	44.0	53.6	69.0	59.3	.93
August.....	84.7	82.0	66.2	42.0	50.4	64.7	50.5	.69
September.....	87.2	74.7	58.5	31.8	42.3	65.7	49.0	.09
October.....	81.0	69.0	47.0	22.0	34.0	77.6	63.8	1.50
Average.....	85.7	70.6	56.6	30.8	42.6	69.4	56.1	-----
Total.....	-----	-----	-----	-----	-----	-----	-----	10.17
Normal:	-----	-----	-----	-----	-----	-----	-----	-----
April.....	-----	59.7	45.5	-----	31.5	68.3	52.3	2.09
May.....	-----	67.8	54.1	-----	40.4	69.0	56.6	2.85
June.....	-----	78.1	63.3	-----	48.2	69.5	57.9	1.62
July.....	-----	83.3	68.3	-----	53.4	71.4	61.2	1.80
August.....	-----	83.0	67.2	-----	51.7	72.7	60.9	1.18
September.....	-----	75.4	59.1	-----	43.0	74.1	60.8	1.26
October.....	-----	63.8	47.6	-----	32.2	74.8	59.5	1.17
Average.....	-----	73.0	57.9	-----	42.9	71.4	58.5	-----
Total.....	-----	-----	-----	-----	-----	-----	-----	11.97

GENERAL APPEARANCE AND COLOR OF FLESH

Freshly cut horizontal sections of mature beets show a peculiar zonation produced by concentric rings of alternating vascular tissue and parenchyma with the sharpest line of demarcation in the region of the cambium. (Pls. 1, 2, and 3.) The distinctness of this zonation varies greatly in different beets and appears to be bound up with color differences of the flesh. Our cultivated sugar beets have a whitish or ivory-colored flesh which, in poor types, is watery greenish or yellow in hue. Beets with a pure-white flesh usually show the least marked zonation, since the vascular tissue merges imperceptibly with the interzonal parenchyma. Such beets give the impression of having very narrow vascular zones separated by broad bands of parenchyma. However, when thin sections of these beets are viewed

against a black background, the width of the vascular tissue will often prove to be much greater than it appeared to be at first glance. Pseudonarrow vascular zones are occasionally found in beets with an ivory-white flesh, but the phloem usually contrasts strongly with the adjacent parenchyma and only the xylem merges imperceptibly into the surrounding tissue. (Pl. 3, C.) Occasionally both phloem and xylem are indistinct, whereas the cambium region stands out as a sharply delimited white or yellowish line. In general, however, color differences suffice to set off the vascular tissue from the surrounding parenchyma. Contrary to general belief, the parenchyma tissue is commonly restricted to a very narrow concentric zone. Within the vascular ring the individual bundles, because of their dark color, contrast strongly with the adjacent parenchyma (pl. 2, B), while xylem and phloem are separated by the white line previously mentioned. It is because of this contrasting coloration of the cambium that what appear as rings when casually observed are in reality made up of the elements of two adjacent rings with a band of interzonal parenchyma in the middle. Flesh color and ring prominence may be correlated with percentage of sucrose, while very broad and prominent vascular rings are typical of certain selections and aid in their characterization.

RING NUMBER AND DENSITY

The rings or annular zones of growth referred to above are more or less equidistant except near the periphery, where they are very close together. The number of the mature rings more or less widely spaced is about five, and this comprises approximately one-half of the total number of rings formed.

Schindler and Proskowetz (10) counted the rings in Klein Wanzleben, Améliorée, and Vilmorin Rose, and found that Améliorée had a consistently smaller number of rings than the other two varieties. Ring counts on beets were also reported by Janasz (6, p. 951), who, however, came to the conclusion that beets behave as individuals, each with its own characteristic ring number.

The data reported in this paper show that in some selections, for example in No. 3956-24 (Table 3), the ring number is consistently and conspicuously high, whereas in others, notably Nos. 1228s-25 and 2287-24 (Table 3), it is characteristically low. There was a considerable amount of variation in the number of total and mature rings in the selections studied, but in general the variation appeared to be less in individuals of a given selection. A high total ring number is frequently correlated with a high mature number, i. e., in a beet with 12 rings 7 may be mature, but occasionally beets with a high mature number have a comparatively low total. Individual beets of the latter type were found in all selections.

Ring determinations on beets of different weight and diameter fail to show definite relationships. Beets of large diameter will occasionally give a high ring count, but so will small-sized beets. In this respect the different selections behave alike. Kraus (?) made similar observations on Klein Wanzleben and Imperial varieties. He found that a decrease in the diameter of the beet is not followed by a decrease in the number of rings, and that beets of the same variety which have an equal diameter may have, nevertheless, a different number of rings.

The width of the three inner rings varies but little; the fourth ring is usually narrower, and the fifth appreciably so. In selections with a small central core the width of the first ring is usually less than that of the two succeeding rings. Table 5 gives individual ring measurements for three beet selections.⁴ The beets are all arranged in a series. Those of the first group (selection No. 554-24) are arranged from low to high percentage of sucrose, those of the second group (selection No. 150-24) from low to high weight, and those of the third group (selection No. 7794-24) from small to large diameter, as measured in the neck region. Ring width appears in many cases to be correlated with weight, and there is a slight relationship between ring width and ring number. A negative correlation is frequently observed in beets with a low ring number; i. e., beets with only 8 rings will have no wider rings than beets with 10 or 12 rings. This condition is probably due to the unbalanced ratio between mature and peripheral rings, caused by a reduction in the number of peripheral but not mature rings. Occasionally, however, the number of mature rings is also reduced, and in such instances the rings are very wide. Beets of this type were frequently observed in selection No. 2287-24 (Table 1). Of more frequent occurrence are selections with relatively narrow rings. In such beets the number of mature rings is 6 and frequently even 7.

TABLE 5.—*Individual ring measurements in three mature beet selections grown at Fort Collins, Colo., 1927*

Item	Core width	Mature ring widths					Immature rings	
		No. 1	No. 2	No. 3	No. 4	No. 5	Number	Width
Selection No. 554-24 (arranged in series from low to high sugar content)-----	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>		<i>Mm.</i>
	2.5	7	6	6	5	4	5	4.5
	2	5	7	7	7	3	4	3.5
	1.5	8	7	6	5	3	6	6.5
	2	8	8	7	7	4	5	8
	1.5	5	6	5	6	5	5	10.5
	6	7	7	7	7	4	5	5.5
	4	8	10	7	7	3	3	3
	3	6	6	6	5	5	6	7
	1.5	5	8	9	8	7	5	7.5
	2.5	8	8	8	7	4	6	4
	3	7	9	6	5	4	7	8.5
	3	7	10	8	7	6	4	6
	4	7.5	10	10	8	5	5	5
	2.5	7.5	9	9	7	3	4	4
	10	10	9	6	6	5	3	5
	1.5	6	8	8	7	6	5	11
	2.5	8	9	8	7	6	4	6.5
	4.5	8	10	8	6	3	4	5
	3	7	8	9	8	6	5	9
	3	9	10	9	8	5	3	4.5
	4	7	8	8	7	4	4	3.5
	3.5	5	6	14	4	6	3	8
	4	9	8	8	7	6	3	12
	5	10	10	10	6	6	6	10
	2.5	9	10	10	8	6	5	7.5
Mean-----	3.0	7.3	8.3	8.0	6.6	4.8	4.6	6.6
Standard deviation-----	1.14	1.42	1.4	1.85	1.04	1.21	1.1	2.48
Coefficient of variability-----	38.20	19.45	16.87	23.12	15.76	25.21	23.91	37.6

⁴ Originally 9 groupings were made, but since the 3 selections behaved more or less alike, 3 groups were selected and condensed into 1 table. Additional data for each are given in Table 1.

TABLE 5.—*Individual ring measurements in three mature beet selections grown at Fort Collins, Colo., 1927*—Continued

Item	Core width	Mature ring widths					Immature rings	
		No. 1	No. 2	No. 3	No. 4	No. 5	Number	Width
Selection No. 150-24 (arranged in series from low to high weight).....	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>		<i>Mm.</i>
3.5	10	8	8	8	5	4	4	4
4	10	10	8	5	3	3	4	5
4.5	10	8.5	7	6	2	4	4	4
7.5	13	11	8	4	2	4	2	2
5	10	8	7	6	4	6	4	6
6	12	9	7	6	3	4	4	4
2.5	9	8	8	6	5	8	12	5
5	10	8	9	7	4	4	4	5
3.5	11	9	8	6	4	6	8	5
6	11	8	7	6	4	6	4	4
7.5	14	9.5	7.5	6	4	5	7	4
5	12	10	8	5	3	5	4	4
5	11	8	8	6	3.5	6	7.5	5
5.5	10	8	8	7	5	7	10	5
5.5	13	9	7.5	7	5.5	5	8.5	5
7	14	11	9	7	5	4	5	5
6	11	9	9	7	6	5	6.5	6
5.5	10	8.5	8.5	7	5	4	5	5
6.5	12	9.5	7.5	5	2	3	2.5	6
9	15	10	8	6	4	5	6	6
3.5	10	10	9	7	6	6	6	6
5.5	11	9	8	7	6	8	12	5
7	13	11	9	8	5	5	5	5
4	11	10	10	7	4.5	5	4.5	5
9	16	9	10	7	6	6	8	8
Mean.....	5.6	11.6	9.2	8.2	6.2	4.3	5.2	6.1
Standard deviation.....	1.69	1.65	.99	.84	.91	1.17	1.26	2.53
Coefficient of variability.....	30.18	14.22	10.76	10.21	14.67	27.21	24.23	41.47
Selection No. 7794-24 (arranged in series from small to large neck diameter).....								
8	13	10	8	6	4	4	5	5
6	15	10	8	5	4	6	4	4
3	9	8	7	6	5	5	5	5
5	12	9	9	9	6	5	6	6
4	11	10	8	7	5	6	8	8
7	10	9	8	5	5	4	6	6
3	10	7	6	5	3	6	7	7
5	11	8	9	8	7	7	15	7
4	8	8	8	7	5	7	6	6
3	8	9	7	5	5	7	10	7
4	12	9	9	8	6	6	8	8
6	10	8	6	6	5	8	10	7
8	15	12	10	9	6	7	2	2
5	12	10	8	6	5	4	2	2
5	11	10	8	6	4	5	6	6
7	11	9	9	7	6	7	9	9
4	10	8	8	6	3	4	4	4
4	11	10	8	6	4	5	4	4
3	11	10	8	6	4	4	4	4
5	11	9	8	8	5	7	10	10
11	15	9	10	7	6	6	8	8
5	11	7	6	5	3	6	5	5
4	11	8	5	5	3	5	5	5
10	14	9	8	6	4	8	6	6
4	9	5	5	5	3	10	4	4
Mean.....	5.3	11.2	8.8	7.8	6.4	4.6	6.0	6.7
Standard deviation.....	2.11	1.89	1.35	1.31	1.23	1.16	1.48	2.67
Coefficient of variability.....	39.81	16.87	15.34	16.79	19.27	25.22	24.67	39.85

Since ring width and ring number are readily measured characters, they attracted the attention of earlier investigators. Bolsunow (2) studied the structure of a collection of 109 sugar-beet varieties and noticed that in the Asiatic varieties the rings were uniformly narrower than in the American varieties. Vivien (13) observed that rich beets had narrower rings and a larger number of them than poor beets, and before him Hoffmann (5) recorded observations showing that

within certain limits the width of the rings varied independently of the sugar content and that in especially rich beets the width of the mature rings varied between 3 and 6 mm.

While it is undoubtedly true that medium or small beets with a conspicuously large number of rings are rich in sugar, it is equally true that beets with an average number of 9 to 11 rings may be either rich or poor. In such cases, however, other characters which will be described later may aid in segregating the poor from the rich forms.

While working on the development of the different tissues of the beet, Seeliger (12) also made observations on the relationship between ring density and sugar content. He defined ring density as the quotient obtained by dividing the total number of rings by the radius of the beet measured in centimeters. He gave the ring density for beets of different ages and sizes and also noted that the ring density did not change after the beet reached a radius of 2.8 cm. Seeliger suggested the use of ring-density measurements as a supplementary selection method in beet-breeding work.

More extensive data on ring density were published by Pack (8). After analyzing the measurements on 500 beets he concluded that there exists a small positive correlation between ring density and percentage of sucrose and a negative correlation between ring density, weight, and total sucrose.

The ring-density coefficient should be a more accurate index of percentage of sucrose than either ring number or ring width, since it takes cognizance of both these variables. Unfortunately, ring number and ring width show only a general and inconsistent relationship to percentage of sucrose, which explains why rich beets occasionally have a low ring-density coefficient and poor beets an abnormally high one. In general, it may be assumed that a high ring-density coefficient is indicative of high sugar trend, and if polariscopic analysis fails to verify this conjecture one must look for other characters, viz, color of the flesh and composition of the vascular tissue, to explain the contradiction.

THE FIRST OR INNERMOST RING

Since the mature rings are more or less evenly spaced, differences in width, unless actually measured, are not readily noticed. Width differences of the first ring, however, easily catch the eye, since the ring is centrally located and can be viewed in its entirety without confusion with other rings. For example, in comparing the measurements of beets from the two selections Nos. 2287-24 and 554-24 (Table 1), the fairly consistent ring-width difference becomes noticeable at once, permitting fairly complete separation of these selections by this character alone.

Selections which are characterized by a large first ring have commonly fewer rings than selections in which the first ring is small or of medium size. That this does not always hold true can be seen by comparing selections Nos. 8753-24 and 7794-24. (Table 3.) Although the ring size is the same in both selections, the average ring number is only 9 for the first and 10.8 for the second group. Individual variations within a selection are common, but they can most often be associated with differences in the size of the beets. There is a tendency for large-sized beets of a selection to have a larger first ring

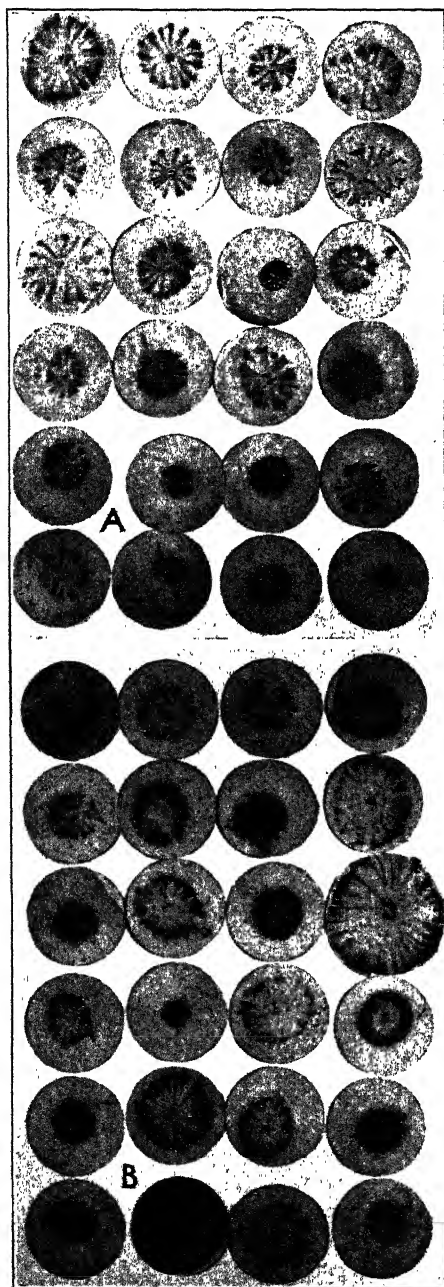


FIGURE 1.—Cross section from central cores of sugar beets, arranged according to weight from left to right with first section from lightest beet. $\times 1\frac{1}{2}$. A, Selection No. 150-24; B, selection No. 2287-24

than small or medium-sized individuals, though occasionally the opposite is true. In selections with a typical small-sized first ring the influence of differences in beet size and ring width is not so noticeable, and very large individuals with a very small first ring are frequently seen.

Definite relationships between the size of the first ring and the percentage of sucrose can not be determined unless the difference in number of rings is taken into consideration; but although it would be expected, *a priori*, that of two beets possessing a large first ring the one having the larger total ring number would be richer in sugar than the one having fewer rings, the measurements recorded in Tables 1 and 2 failed in most cases to confirm this assumption.

THE CENTRAL CORE

The central core forms a 2-lobed, more or less star-shaped structure of which the innermost part constitutes the primary xylem plate. It is most uniform in the neck region, its symmetry being later broken by the passing out of radial strands of vascular tissue—the root traces. Serial sections through a beet from the neck region to the taproot end show that the size of the central core varies somewhat, being usually smaller in the taproot end. Occasionally the central core will be considerably wider in the neck region, which is especially true of broad, rapidly tapering beets. To avoid injury to the beets Hoffmann (5) examined the central core at the taproot end primarily.

He found that the anatomical structure of the central core in this region is very symmetrical and well suited for comparative studies.

The data obtained in the present studies indicate that the measurements of the central core are of practical value in the anatomical characterization of these selections, since size differences of the core are easily determined and compared. The majority of the selections studied have a medium-sized core, but certain selections stand out preeminently because of their very large or very small cores, as can be seen from Figures 1, 2, and 3. The degree of uniformity of core size differed in the selections studied. In some, notably those having a very small core, the uniformity is striking (fig. 3, B), and the same is true, though to a lesser degree, of some of the large-core types. On the other hand, there are some selections which at present can not be placed in any group, since the variation in core size is too marked. The size of the central core is slightly influenced by the diameter of the first ring. In selection No. 2287-24 (Table 2), where the individuals have a uniformly large first ring, the central core is also large. Likewise in selections No. 554-24 (Table 2), where the first ring is small, the core is also greatly reduced. Judging from the material used, the size of the central core and the weight of the beet do not seem to be related to each other, and there appears to be no relation between the size of the core and the percentage of sucrose. These facts are evident from an examination of Figures 1, 2, and 3, in which the individual photographs are arranged in an ascending series from low to high percentage of sucrose. In short, the size of the central core is a definite character apparently characteristic of a type, and the indications are that it will form a valuable morphological character for type description rather than for sucrose percentage.

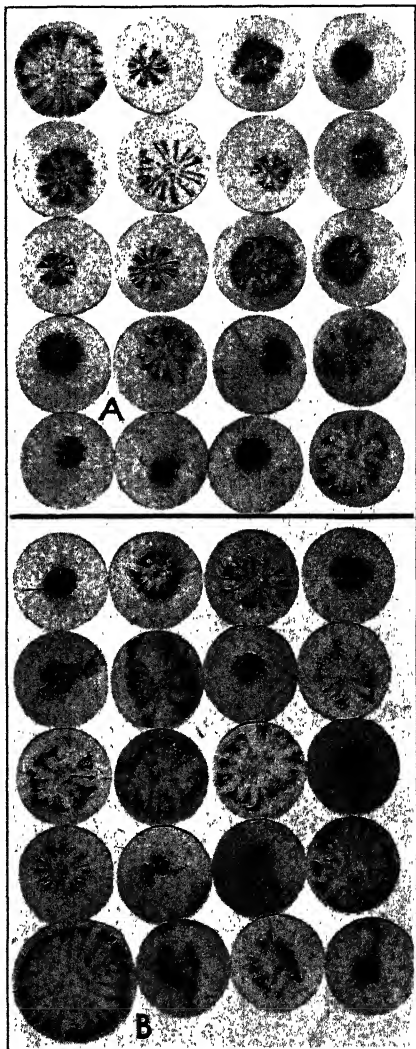


FIGURE 2.—Cross sections from central cores of sugar beets, arranged according to weight from left to right with first section from lightest beet. $\times 1\frac{1}{2}$. A, Selection No. 1612-24; B, selection No. 1427-24

The vascular tissue of the central core is composed of numerous bundles which converge toward the central xylem plate. In large cores these bundles or rays terminate some distance from the center,

and only a few scattered elements extend farther in. The number of core rays is somewhat larger at the taproot end of the beet than in the neck region (Table 6), although the actual size of the core is commonly smaller at the taproot end. The size and number of the core rays vary, though in certain selections there is a fair degree of uniformity. In selection 1228s-25 (fig. 4, A) the rays are uniformly narrow and very numerous. Sometimes the rays are so close together that the bundles coalesce and one sees only numerous xylem rows embedded in small-celled parenchyma. The number of rays is not bound up with the size of the core (fig. 5, A, B, and fig. 6, A), although large cores often give a greater peripheral count because of the splitting off of secondary rays, which, however, do not reach the center. Occasionally the number of core rays is very small, as is shown in Figure 4, B. The absence of a readily seen relationship between the number of rays and the weight and percentage of sucrose is shown in Table 6. As in Table 5 the beets are arranged in series; those of the first group (selection No. 898-24) are arranged from low to high percentage of sucrose, those of the second group (selection No. 1569-24) from low to high weight, and those of the third group (selection No. 7794-24) from small to large neck diameter.

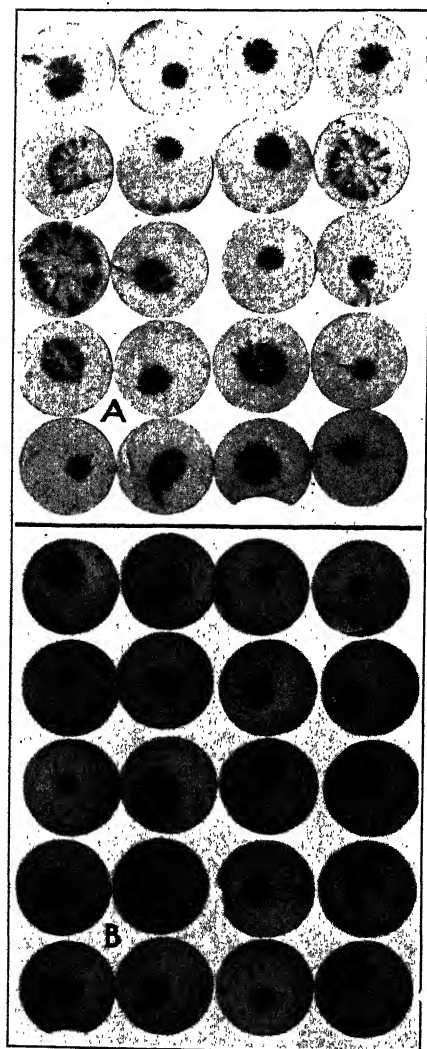


FIGURE 3.—Cross sections from central cores of sugar beets, arranged according to weight from left to right with first section from lightest beet. $\times 1\frac{1}{2}$ A, Selection No. 2445s-25; B, selection No. 554-24

eter. Originally nine groupings were made, but since the different selections behaved alike, only the data of three groups are shown. (Table 6.)

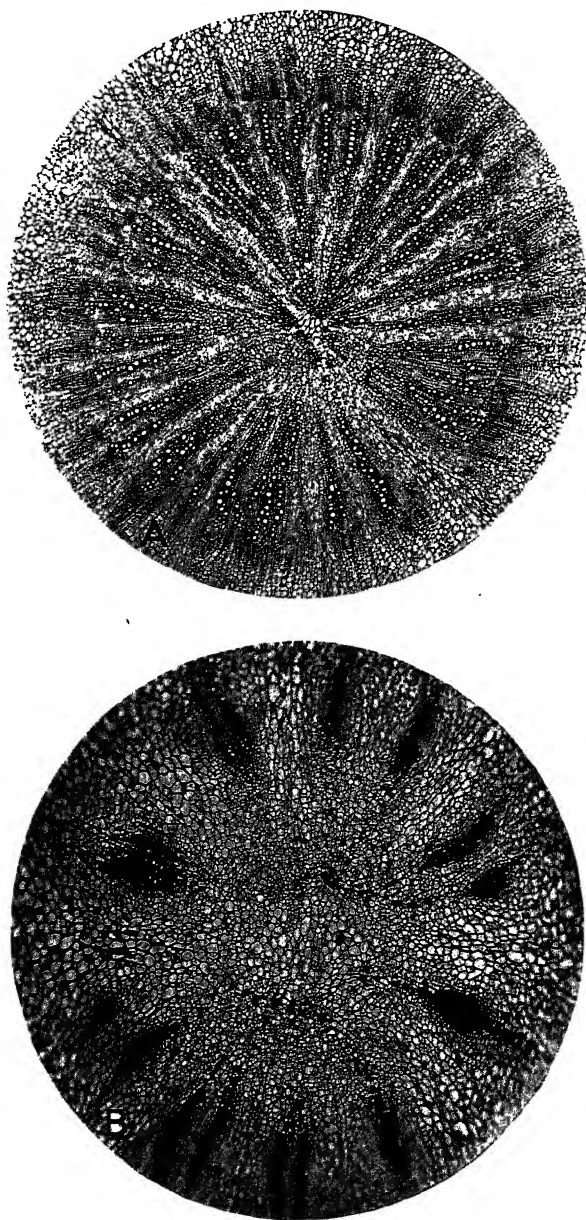


FIGURE 4.—Cross sections of cores of sugar-beet selections. A, No. 1228s-25. $\times 16$. B, No. 1845s-25. $\times 10$. Note the radical difference between the structure of the two cores

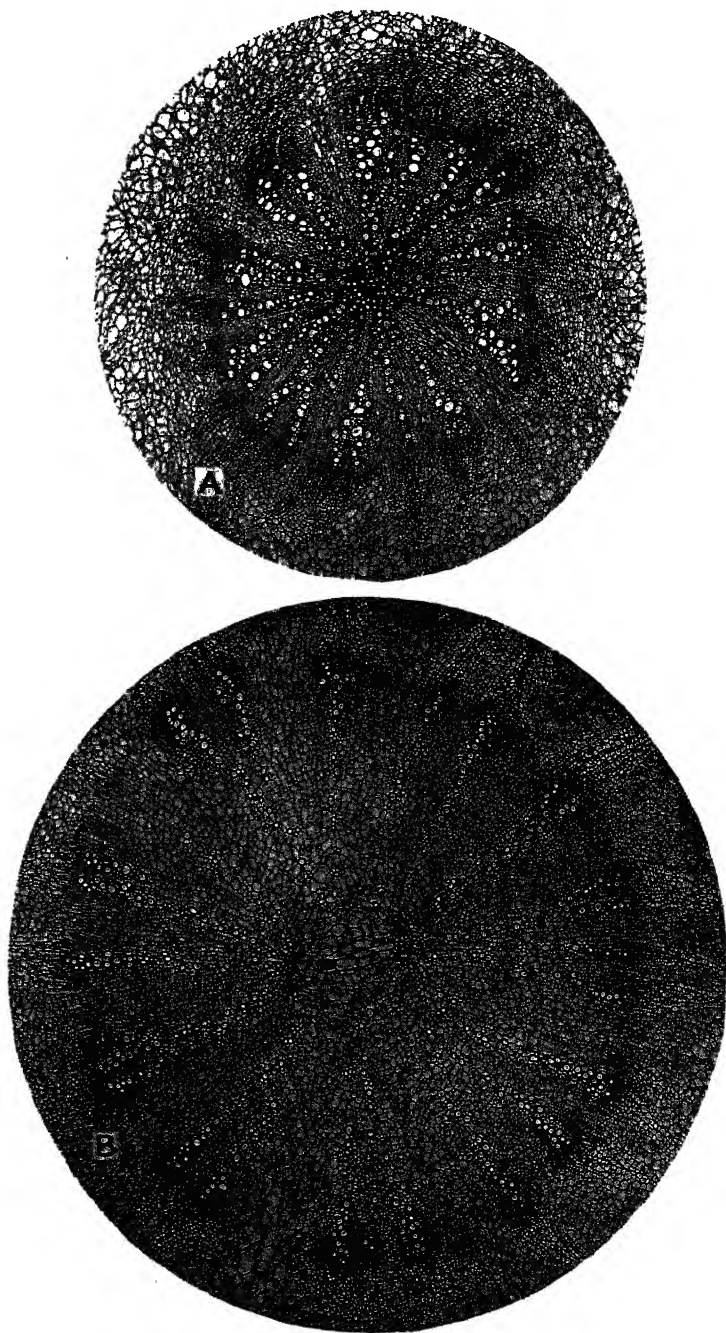


FIGURE 5.—Cross sections of two sugar beets of selection No. 18458-25 with 15.8 per cent sugar content. 19. A, Small dense core; B, larger, more or less open core. The bundles are very symmetrical and have well-developed sheaths

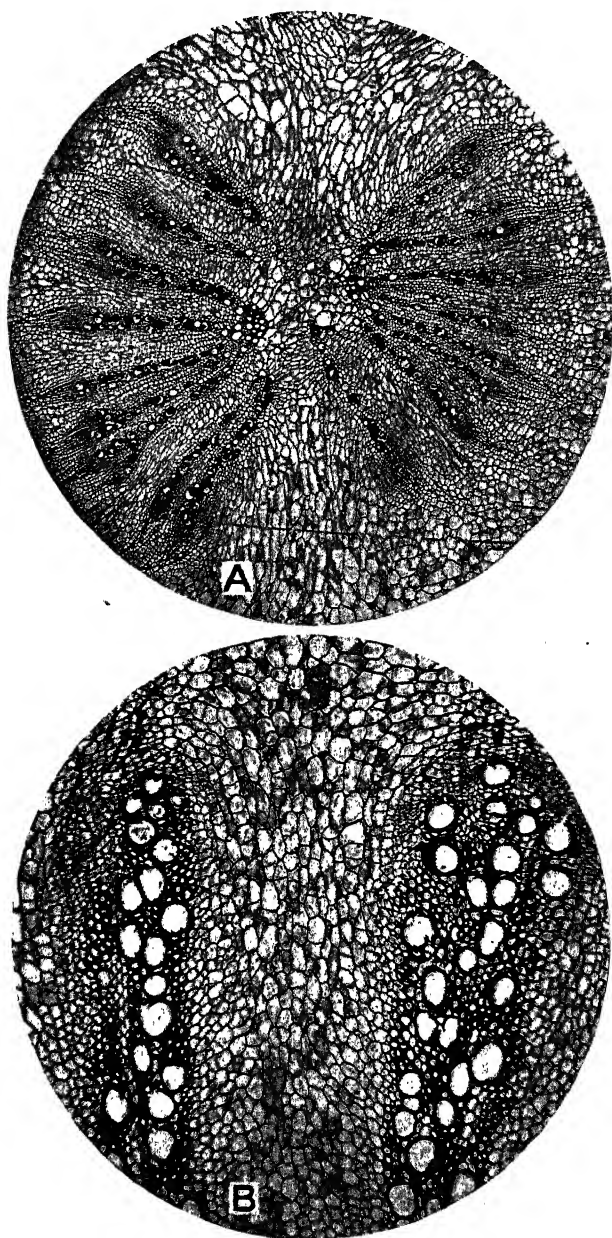


FIGURE 6.—Cross sections of sugar-beet selections. A, Central core of No. 18458-25 with 11.2 per cent sugar content. Note the narrow, very symmetrical rays. $\times 16$. B, Bundle of central core of No. 4621-22 with most of the sugar sheath lignified. $\times 63$

TABLE 6.—Vascular strands of core rays in central core of three beet selections grown at Fort Collins, Colo., 1928

Item	Neck end		Tap end		Item	Neck end		Tap end	
	Diam-eter of core	Rays	Diam-eter of core	Rays		Diam-eter of core	Rays	Diam-eter of core	Rays
Selection No. 895-24 (arranged in series from low to high sugar content)-----	Mm.	Num-ber	Mm.	Num-ber	Selection No. 1569-24.	Mm.	Num-ber	Mm.	Num-ber
8	13	5	17		6	15	2	19	
7	12	5	16		3.5	13	3.5	18	
3.5	13	5	13		4	11	4	18	
5	16	2	14		6.5	16	3	17	
5	8	4	13		6	13	3	21	
5	15	3	13		5	15	3	18	
5	13	5	16		4	15	3	18	
3	13	4	14		4	14	2	15	
4	14	3.5	16		7	18	3	23	
3.5	13	6	15		4.5	11	2.5	19	
4	14	4	15		4	11	4	15	
3.5	13	3	21						
4	17	3	17		Mean-----	4.4	13.9	2.9	18
3.5	14	4	16		Standard devi- ation-----	1.29	2.18	.58	2.54
4	11	5	17		Coefficient of variability---	29.32	15.68	20.00	14.11
3	8	4	14		Selection No. 7794-24 (arranged in series from small to large neck diameter)-----				
5	15	5	22						
4	13	4	15		2.5	10	3.5	14	
5	18	4	16		3	19	4	19	
5	15	5.5	20		2.5	18	3.5	19	
6	16	4	19		3	10	3	13	
5	11	4	15		4	10	3.5	14	
6	12	6	16		3.5	16	4	22	
4	10	4	12		3.5	12	4.5	20	
3.5	12	2	18		6	16	4	18	
4	11	4	17		4.5	15	5	20	
Mean-----	4.6	13	4.2	16	3	9	3	15	
Standard devi- ation-----	1.4	2.30	1.03	2.47	2.5	16	3	18	
Coefficient of variability-----	30.43	17.69	24.52	15.44	2	15	2	17	
Selection No. 1569-24 (arranged in series from low to high weight)-----					3	17	5	22	
					2	16	3	20	
4	18	3	22		4	15	3.5	19	
5.5	19	4	20		4	13	3.5	16	
3.5	13	2	18		5	22	5	25	
2.5	15	3	18		4	17	4	17	
5.5	14	3	15		4	11	4	16	
5	13	2.5	19		2.5	12	3	16	
3	12	2.5	16		2	15	2	22	
3	12	3	14		6	17	4	19	
6	11	3.5	17		2.5	14	3	15	
3	14	2.5	19		3.5	11	3.5	16	
3	13	2.5	19		Mean-----	3.3	13.8	3.4	17
6	14	3	16		Standard devi- ation-----	1.16	3.21	.82	2.93
3	15	3	16		Coefficient of variability---	35.15	23.26	24.11	17.23
4	12	2	22						

STRUCTURE OF THE VASCULAR RING

The vascular zone of each ring is composed of numerous collateral bundles separated from one another by medullary-ray tissue of varying width. Secondary rays frequently develop inside the bundles, causing them to split and making it difficult, in bundle counts, to obtain a correct estimate of the number of bundles per unit of distance. In longitudinal section the bundles of a ring anastomose tangentially, forming a network of unequal meshes. (Fig. 7, A-C.) Furthermore, Dostál (3), in a recent study on tangential polarity in beets, has shown that the course of these anastomosing bundles is somewhat dextrorse for the outer rings, sinistrorse for the inner rings, and straight radial for the intermediate rings. This asymmetrical ana-

tomical development, according to Dostál, is reflected externally by the somewhat spiral course of the lateral beet depressions containing the root traces.

The relative width of the vascular tissue was determined from freshly cut sections, because of the color contrast which the latter afford and which disappears in preserved material. An examination of a number of individual selections indicates that vascular prominence or conspicuousness is a property of a selection and is independent of beet size. There will, of course, be a variation in the

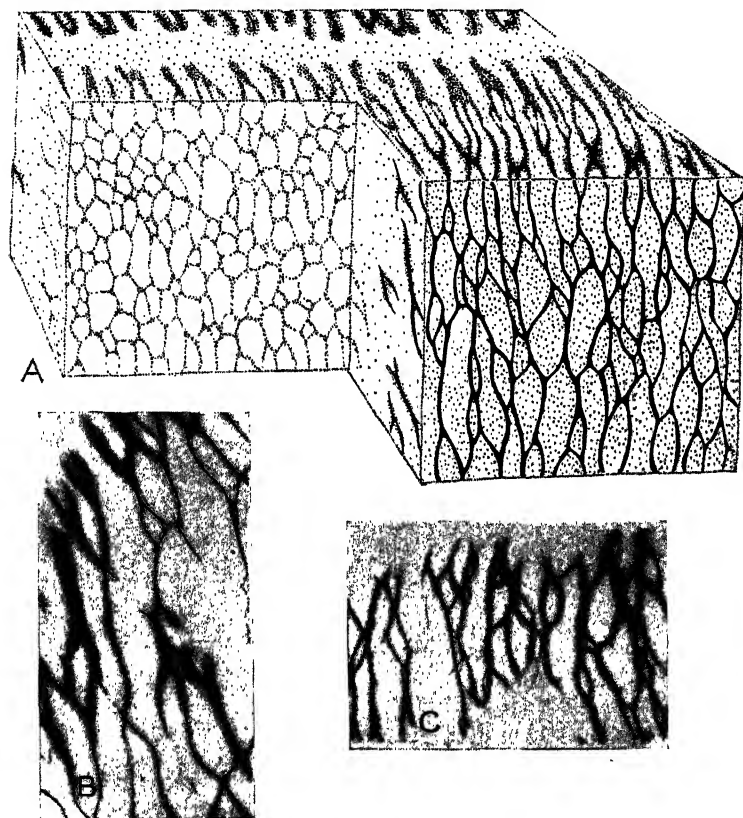


FIGURE 7.—Anastomosing of vascular region of sugar beet. $\times 2$ A, Drawing of fourth ring; B, photograph of tangential section through xylem; C, similar section from a different part of the beet

width of the vascular rings of large and small beets, but the relative proportion between width of vascular tissue and width of interzonal parenchyma will remain constant. Vascular-ring prominence and percentage of sucrose do not seem to be related. A broad vascular ring associated with narrow interzonal parenchyma is not necessarily indicative of high sugar content, for sometimes individual beets with inconspicuous rings have a higher percentage of sugar than beets with prominent rings, a fact well illustrated in Plates 1, B, and 2, B. It must be admitted, however, that in this case the beet illustrated in Plate 1, B, had a higher ring-density factor than the second beet.

MICROSCOPIC CHARACTERS

Comparative anatomical studies on the structure of the sugar beet date back practically to the beginning of beet culture, though the most extensive phytotomic studies were made at the close of the nineteenth century at the various sugar-beet breeding stations.

Schindler and Proskowetz (10) in their pioneer studies on beet races also made an investigation of the anatomy of three races—Klein Wanzleben, Vilmorin Rose Hâtive, and Vilmorin Blanche Améliorée. They determined for 10 uniform individuals of each race the number of rings, the number of vascular bundles, and the size of the interzonal parenchyma cells. The counts were made in the peripheral region of the neck, and the size of the area studied had a radial and tangential extent of 1 cm. and 0.5 cm., respectively. Although counts were made at three different places, the areas involved were too small to yield reliable data, so that the observational error could easily have been big enough to account for the differences in the data obtained. Schindler and Proskowetz concluded that the beets rich in sugar possess a larger number of lignified bundles, and therefore more lignin, and a smaller celled interzonal parenchyma than beets poor in sugar.

Schneider (11), questioning the results of Schindler and Proskowetz, maintained that the number of xylem-containing bundles is no index to the amount of lignin, and that Schindler's method of counting the bundles is misleading, since bundles that had only a single xylem cell were counted, while those without xylem cells were omitted. Schneider reinvestigated the results of Schindler and Proskowetz, but he used in his studies an even smaller number of beets than they had used. He examined the varieties Vilmorin Blanche and Chotetovka. Like Schindler, he made his counts in the peripheral region of the neck but extended the radial depth of the areas to 1.77 cm. Schneider found that the variety rich in sugar had fewer rings, fewer vascular bundles, fewer but wider and thicker vessels, and fewer lignified parenchyma cells than the variety poor in sugar. According to Schneider, beets rich in sugar are less woody than those poor in sugar, a view that is in harmony with observations of De Vries (14) and opposed to those of Schindler.

TABLE 7.—Total and lignified bundles in rings of Klein Wanzleben and Imperial varieties (according to Kraus)

Item	Number of bundles per unit area in ring No.—									
	1.	2	3	4	5	6	7	8	9	10
Klein Wanzleben:										
Total	8.7	9.6	11.0	14.6	14.6	17.4	17.9	14.3	21.0	23.7
Lignified	7.7	8.4	7.3	8.7	6.1	6.4	6.3	4.1	.7	.2
Imperial:										
Total	8.5	13.0	15.5	18.8	19.7	19.0	20.3	19.6	22.7	26.0
Lignified	7.1	8.0	8.4	9.4	9.0	8.8	5.6	4.7	5.2	-----

Kraus (7) counted the bundles in the different rings of the varieties Imperial and Klein Wanzleben. The counts were made on six individuals of each variety and the width of the areas measured was 2 mm. Table 7 gives the averages of his results for total and lignified bundles. These two varieties show differences in the number of total and of

lignified bundles. In Klein Wanzleben the number of bundles increases centrifugally, while the number of lignified bundles decreases in the same ratio. In the Imperial variety this condition is less pronounced. Kraus is inclined to think that the smaller the number of bundles the greater is the degree of lignification within a bundle.

Since a correlation between the percentage of sucrose and the anatomical structure was the primary object of all phytotomic studies, the localization of the sugar in the different tissues was investigated and special correlative studies of tissues rich in sugar were made. More than 50 years ago Wiesner (15) and De Vries (14) showed that the small-celled parenchymatous tissue surrounding the bundles was especially rich in sugar. This tissue was subsequently named "sugar sheath" by De Vries. The sugar-sheath cells, in addition to being smaller than the surrounding parenchyma cells, have thicker walls. The walls of the sugar-sheath cells are of cellulose and ordinarily do not change even in mature beets, but occasionally they become lignified. Lignified sugar-sheath cells are found individually or in groups, and in extreme cases the entire sheath may be lignified. (Fig. 6.) At a later period Peklo (9), studying the distribution of the sugar by the phenylhydrazine method, discovered that the largest osazone precipitate was found in the sieve tubes, and that therefore the phloem was to be considered the tissue especially rich in sugar. Geschwind (4) examined the development of the sugar sheath in 500 beets and came to the conclusion that while it is possible to separate rich beets from poor beets on the basis of differences in the development of the sugar sheath, subsequent breeding experiments as reported by Hoffmann (5) showed these results to be of no practical significance. The same can be claimed to some extent for the work of Peklo, since he was not always able to correlate massiveness of phloem with sugar content of the individual.

THE FOURTH VASCULAR RING

In examining the bundles of the various vascular rings it is apparent that the oldest, i. e., the innermost ring, has the smallest number of bundles per linear dimension and that the peripheral ring has the largest. In other words, the number of bundles increases centrifugally. Furthermore, it will be noted that in any given ring the spacing of the bundles is not equal and the size of the bundles also shows a certain degree of variation. This is due to the fact that the bundles anastomose profusely tangentially, so that a given bundle will have a different appearance in different sections. To obviate this difficulty it is necessary to count the bundles over a sufficiently large area, and since, because of the great labor involved, it is impracticable to count the bundles in all the rings, it was considered best to make a more complete study of the fourth ring and carry it through for the individuals of all the selections studied. But even then it is impossible to arrive at some simple representative figure, since it is left to the observer to evaluate what is an individual bundle. In some beets the bundles are fairly uniform and distinct, but in others it is often difficult to decide whether a certain bundle is a component of a large bundle or a unit in itself. (Fig. 8.) Even if one succeeds in eliminating as far as possible the personal equation, direct comparisons of bundle counts may be of little value, since some

individuals possess characteristically large bundles, while in others they are small, although quite distinct.

From the data presented in Table 2 it will be seen that each beet has its own number of bundles; however, if the individuals of different groups are compared there appears to be no doubt that in certain selections, for example, No. 3956-24, the number of bundles is consistently low; in others it is much higher. Generally speaking, a large beet has fewer bundles per unit area than has a small beet, but this relation does not always hold true. There appears to be no distinct relation between number of bundles and percentage of sucrose. This

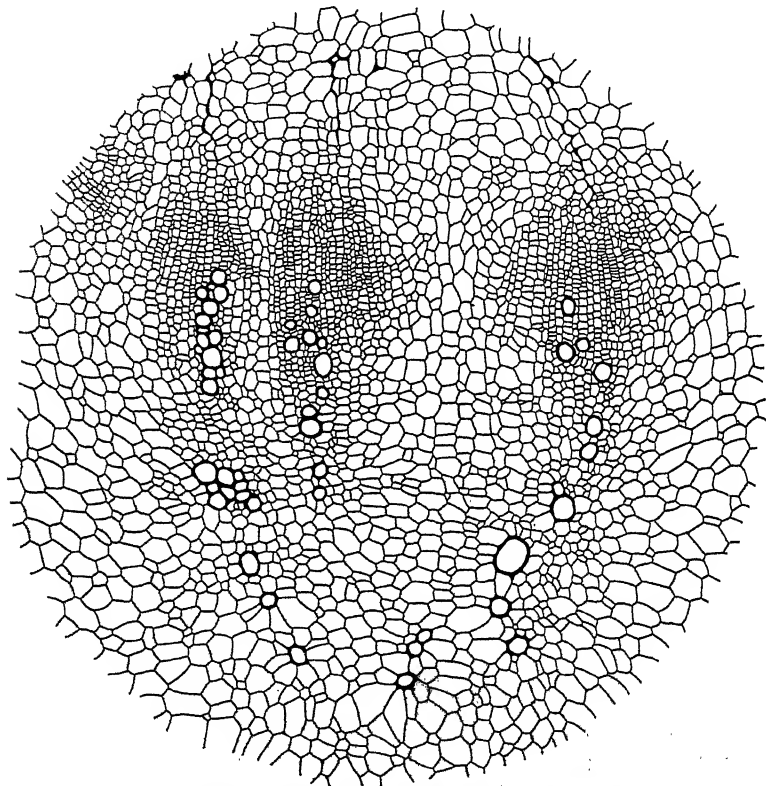


FIGURE 8.—Large bundle from fourth ring of selection No. 2287-24. $\times 70$

is true in individuals of the selection and in individuals of different selections.

The structure of the individual bundles, except for size and degree of maturity, is alike. (Fig. 9.) The bundles are widest in the region of the cambium and taper gradually toward the phloem and xylem poles. This gives them the appearance of a double wedge. (Fig. 10, A.) The xylem is formed of one or several disconnected rows of vessels, surrounded by a sheath of elongated small-celled parenchymatous tissue—the sugar sheath of De Vries. The phloem is composed of sieve tubes, companion cells, and phloem parenchyma, is

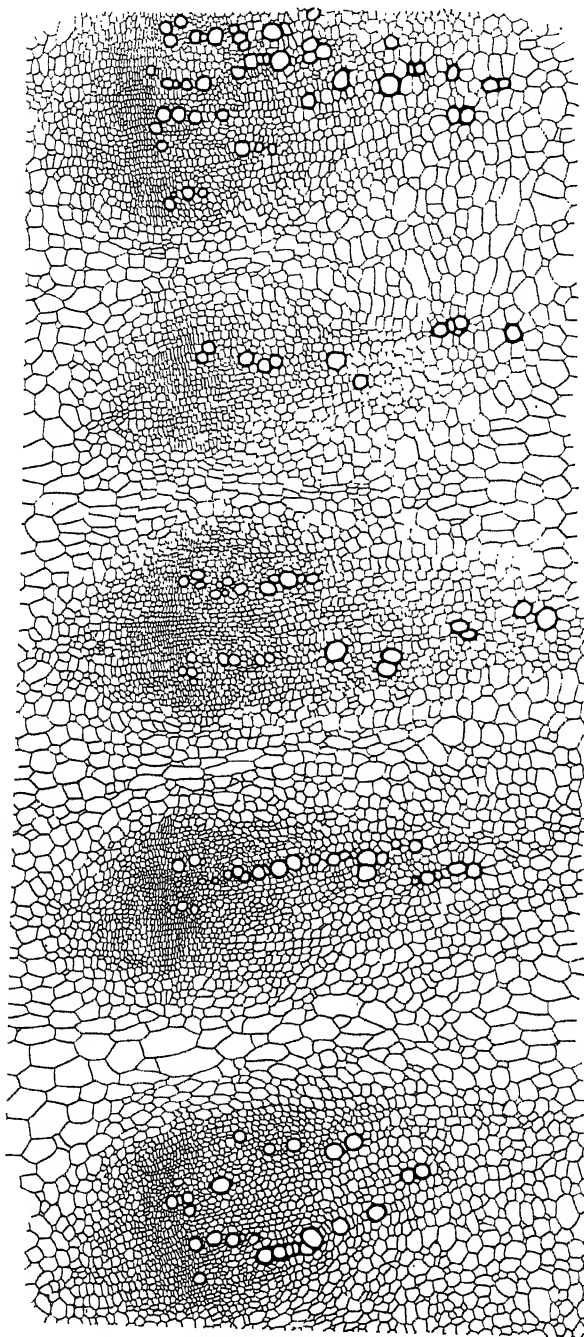


FIGURE 9.—Partial cross section of fourth ring. $\times 85$

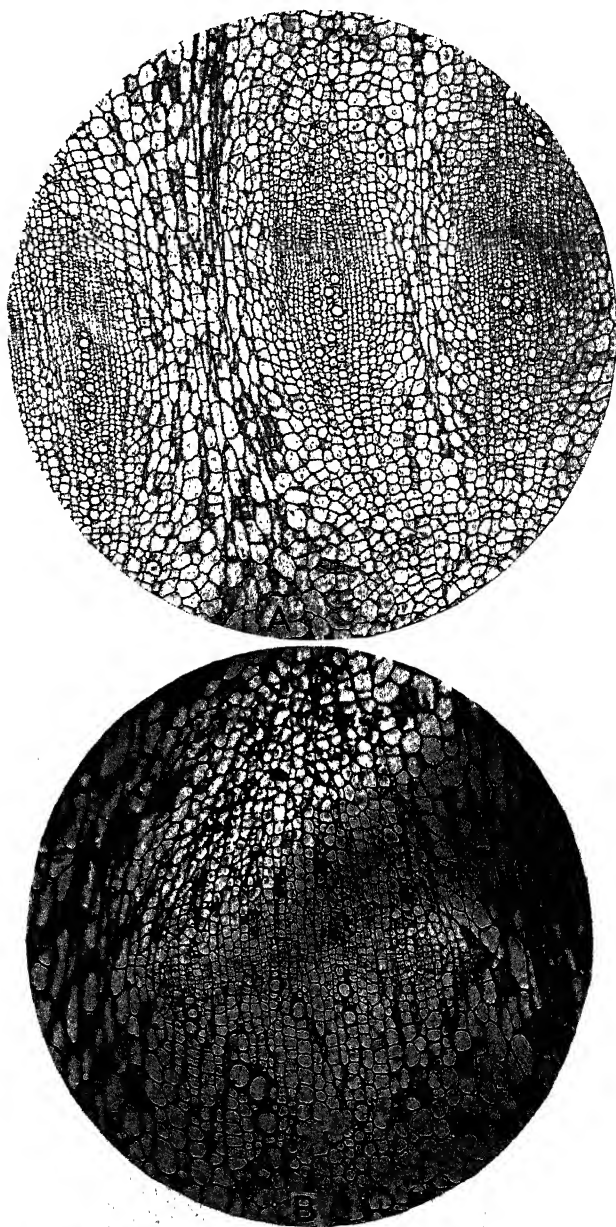


FIGURE 10.—Cross sections of sugar-beet vascular bundles. A, Rich beet of selection No. 4621-22. $\times 45$. B, Small, very rich beet from Rocky Ford, Colo. Note large amount of phloem. $\times 100$

commonly well developed, and exceeds in mass the xylem. This is especially true of the bundles of the more peripheral rings.

The existence of variation in size of the bundles has already been mentioned. The difference in size may extend to either the radial or the tangential dimension. One frequently observes in beets with a low percentage of sucrose a rather elongated xylem pole surrounded by a very narrow zone of sugar-sheath cells. In beets high in sucrose, on the other hand, the sugar sheath is much broader, although the amount of xylem may be less than in the first type. Certain selections, especially No. 3403-24 (Table 2), are characterized by a very narrow vascular ring. (Fig. 11, B.) This same selection also illustrates the fact that narrow-zoned individuals have not necessarily a small number of vessels; in fact, the number in some individuals may be quite large. (Table 8.) The xylem cells of the individual bundles vary normally in size. The cells of greatest diameter are commonly found near the xylem pole and those of smallest diameter near the cambium. In individuals that are low in sucrose, however, there is no reduction in the size of the xylem cells of the cambium region, a fact recorded by many of the earlier investigators. This, however, is not to be considered a hard and fast rule, since in bundles of poor beets small xylem cells may be found in the cambium region; whereas, conversely, bundles in rich beets may have large xylem cells near the cambium. Aside from normal variations of xylem cells within a bundle there is also a general variation in size when different individuals are compared. This is well illustrated in Figure 12. In A, B, and D of that figure the xylem cells appear of the same size, but in C the small diameter of the cells is notable.

TABLE 8.—*Number of xylem cells per centimeter as counted in bundles of fourth ring of sugar-beet selections grown at Fort Collins, Colo., 1927 and 1928*

1927									1928		
No. 8753-24	No. 6008-25	No. 3403-24	No. 1591-24	No. 193 R-25	No. 23108-25	No. 4621-22	No. 24458-25	No. 2287-24	No. 8753-24	No. 6008-25	No. 3403-24
182	296	248	-----	365	198	158	215	263	229	220	235
285	463	211	143	244	274	187	330	183	267	205	148
276	199	-----	135	198	250	231	157	205	165	210	190
306	292	-----	207	218	159	174	220	278	267	149	215
213	166	197	104	243	217	150	170	237	251	167	212
237	280	125	185	334	274	218	300	208	223	240	140
-----	280	-----	149	189	259	383	246	330	186	220	169
255	254	-----	149	160	-----	-----	200	208	200	195	276
324	251	202	176	233	198	191	276	267	228	230	278
230	250	150	170	197	240	215	231	100	179	210	249
247	360	235	143	295	210	-----	154	226	-----	-----	-----
233	202	231	154	309	198	185	182	-----	-----	-----	-----
-----	226	238	99	233	345	-----	178	243	-----	-----	-----
171	332	237	220	378	205	110	293	145	-----	-----	-----
232	190	-----	170	200	131	160	301	140	-----	-----	-----
212	385	178	-----	260	159	216	163	173	-----	-----	-----
138	387	158	-----	242	227	255	198	138	-----	-----	-----
300	237	258	108	201	161	298	140	246	-----	-----	-----
187	300	170	122	300	212	238	210	194	-----	-----	-----
267	235	222	219	236	148	175	233	155	-----	-----	-----
247	249	120	174	-----	232	-----	225	156	-----	-----	-----
180	200	363	180	-----	177	-----	234	282	-----	-----	-----
218	346	-----	216	-----	356	-----	177	150	-----	-----	-----
-----	270	136	177	-----	111	-----	244	198	-----	-----	-----
338	258	186	107	-----	260	-----	182	283	-----	-----	-----
Mean...239	275	203	159	251	216	208	218	204	219	205	211

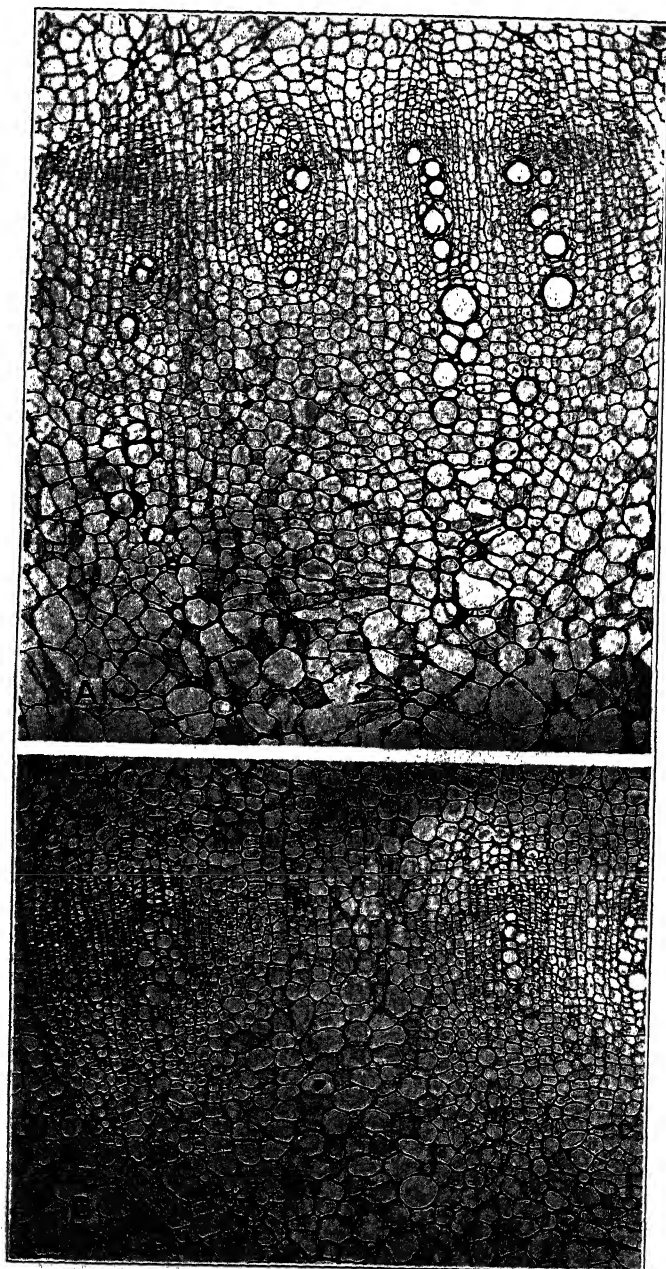


FIGURE 11.—Cross sections of vascular bundles of sugar-beet selections. $\times 50$. A, No. 6942-24 with 11.3 per cent sugar content; B, No. 3403-24 with 14.1 per cent sugar content. Note extreme narrowness of vascular bundles

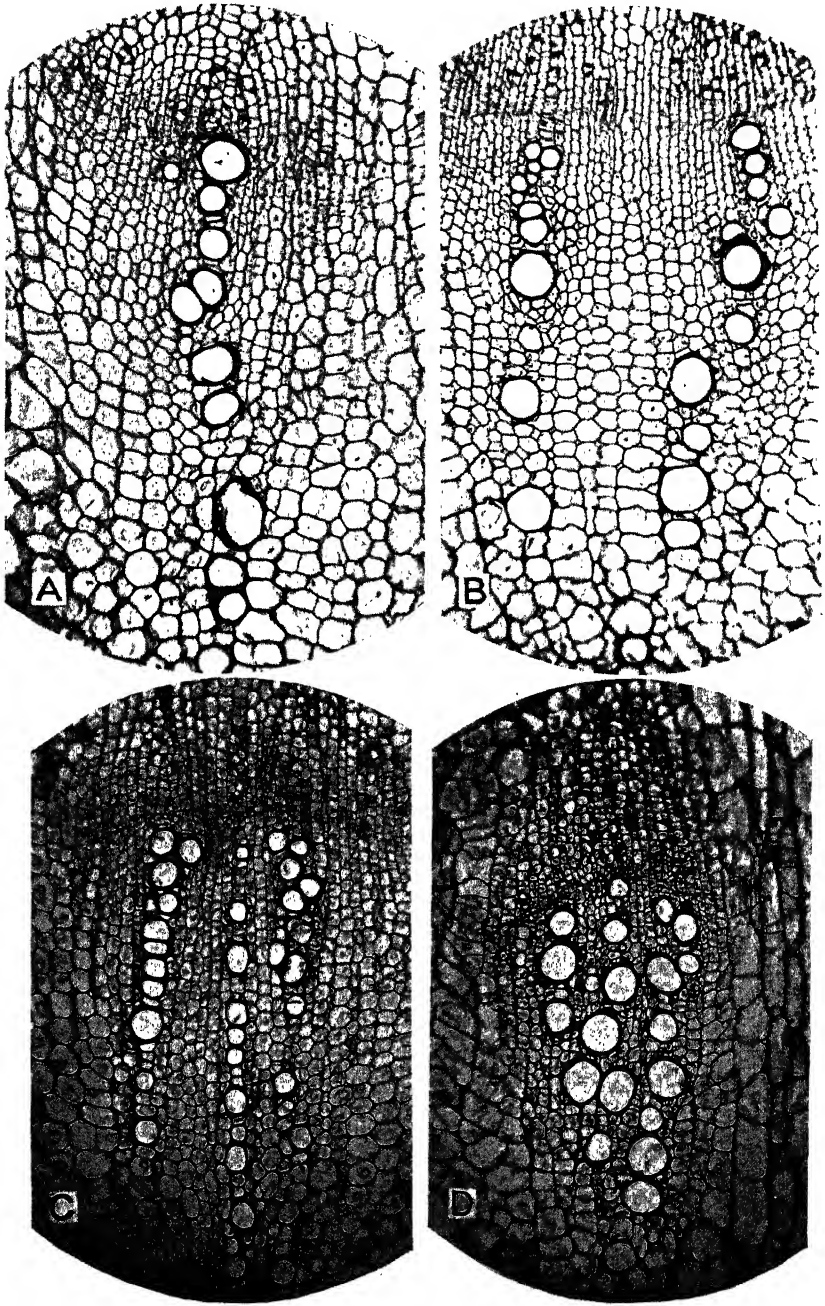


FIGURE 12.—Cross sections of vascular bundles of sugar-beet selections. $\times 95$. A and B, No. 5942-24. Sugar content of A, 11.3 per cent; of B, 13.6 per cent. C, No. 1228s-25; sugar content, 12.1 per cent. D, No. 1161-24; sugar content, 11.8 per cent

In many selections the xylem consists only of vessels of varying sizes, but in some there are also a larger or smaller number of lignified sheath cells. (Figs. 6, B, and 13, B.) These cells appear singly, in small groups, or in large areas. Not all individuals of a selection possess them, and while their presence is not necessarily correlated with sugar content, there is a tendency for rich beets to have a smaller number of these lignified cells than poor beets, or they may be absent altogether in the better types.

Just as the count of vascular bundles is subject to error, an evaluation of the amount of lignified tissue is even more so. In bundles having only a few vessels the size of the individual cells does not differ greatly, and counts in individuals of the same type give at least comparative data. However, in the case of many vessels a count of the actual number is misleading, since many of the "supernumerary" vessels are usually small in diameter and do not contain the amount of lignin found in large vessels. The phloem part of the vascular bundles is usually well developed, which is especially noticeable in the peripheral rings where xylem elements are often still undifferentiated. It would appear from the investigations of Peklo (9) that the phloem is the seat of highest sugar concentration, and its relative amount should therefore be directly correlated with the sugar content; and, indeed, very rich beets, as illustrated in Figure 10, A, have a very massive phloem. The investigations of Peklo have never been repeated, and they are only roughly approximated in connection with the present anatomical studies.

Beets with rather wide vascular zones were cut in slices about one-fourth inch thick and the slices divided along the cambium line of the mature rings. The samples were analyzed separately but showed practically no difference in regard to sugar distribution. It would appear that either the phloem tissue is not so high in sugar as was claimed by Peklo, or, as is more likely, the accumulation of sugar in the phloem is counterbalanced by the sugar stored in the sheath cells surrounding the xylem. It has been shown previously that a wide vascular ring does not always denote a rich beet, but if individuals with a massive phloem should polarize low, one would usually not have to look far to find an explanation.

The vascular bundles of the central core reflect in a large measure the appearance of the bundles of the mature rings. (Fig. 14.) The core bundles are in general more lignified than the mature ring bundles, a condition that is very striking in small compact cores. The vessels of the core have a tendency to be large, especially in individuals with a low percentage of sucrose. In rich beets they are not only of smaller size but suffer a further reduction in the region of the cambium. Geschwind (4), who examined the central core of the taproot end of the beet, noticed the same tendency.

The interzonal parenchyma proper comprises a relatively narrow zone of parenchymatous tissue. Actually, however, the zone seen in looking at cross sections of a beet comprises three regions: A centrifugal, more or less broad band containing scattered xylem cells; an intermediate, purely parenchymatous zone; and an inner region containing obliterated phloem. Since earlier investigations associated a small-celled parenchyma with a high percentage of sucrose, an attempt was made to verify such a correlation in the selections under

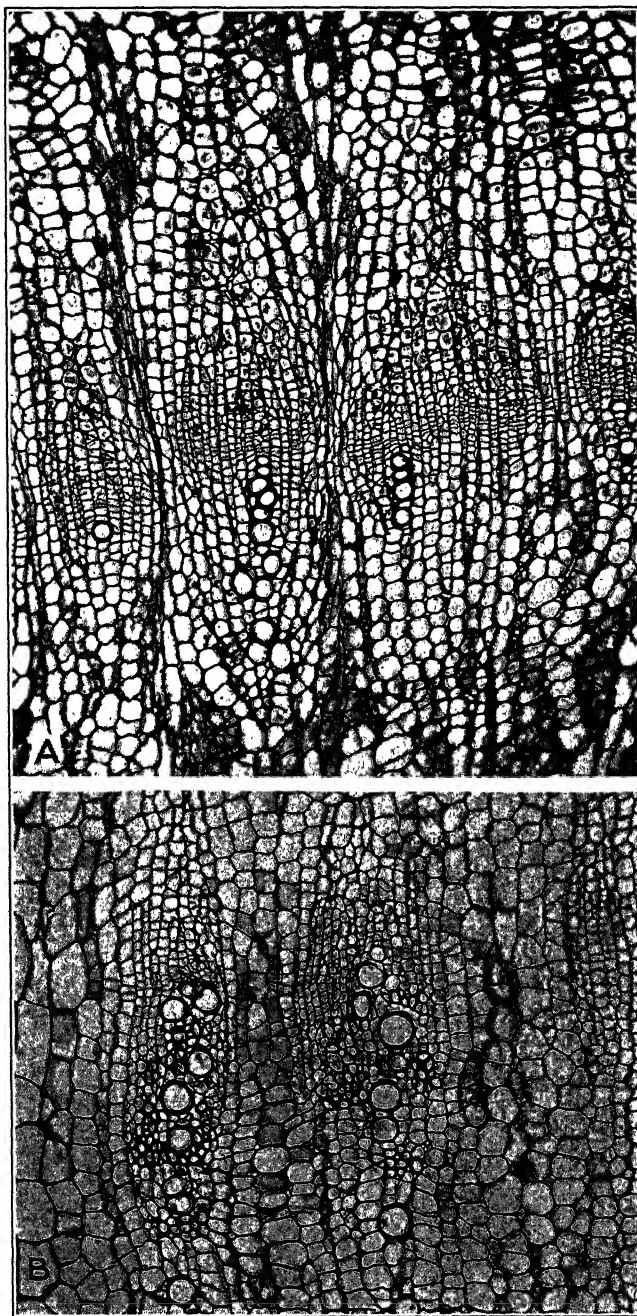


FIGURE 13.—Cross sections of vascular bundles of selections of sugar beets from Rocky Ford, Colo. $\times 110$. A, Very rich beet with phloem large in comparison with xylem. B, No. 1973, with 12.5 per cent sugar content. Note lignified sugar-sheath cells and large size of xylem in comparison with A.

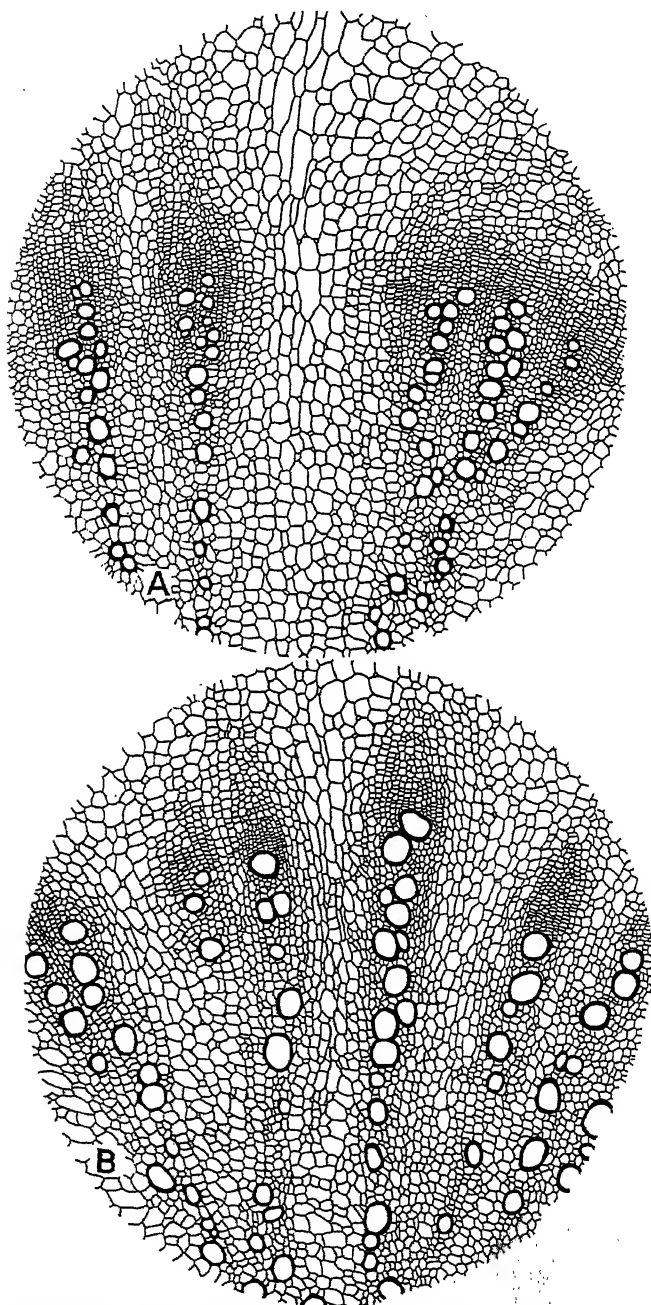


FIGURE 14.—Cross sections of vascular bundles of central cores of sugar beets. $\times 60$.
A, Shown in Figure 5; B, shown in Figure 6, A

observation. It was found difficult to approximate correctly the size of these cells. Individual measurements were considered inadequate, and in counting the cells of a given region one has to contend with errors arising from the false impression made by cells that have been cut through the corners and thus appear small. Everything considered, however, the method of projecting a slide on a white background and counting the cells was found to be most satisfactory. Table 9 gives data on individuals of three selections. The data are arranged in a series from low to high sugar.

TABLE 9.—*Number of interzonal parenchyma cells per square millimeter in fourth ring of sugar-beet selections grown at Fort Collins, Colo., 1927*

No. 4545-24		No. 2445s-25		No. 2287-24	
127	176	95	163	92	83
99	120	96	130	65	56
102	139	101	151	62	60
126	131	176	97	82	83
133	125	144	150	96	115
129	97	97	128	72	87
121	119	156	91	78	133
108	145	99	125	81	137
132	128	183	133	117	76
123	102	183	129	55	94
94	146			71	84
				61	67
Mean...123		Mean...131		Mean....83	

While there is a great deal of variation and no apparent connection between the size of parenchyma cells, the weight of the beet, and the percentage of sucrose, the large-celled parenchyma in No. 2287-24 is related to the large central core and broad first ring of this selection, and it is not unlikely that some of the other selections might show similar relationships.

EFFECT OF ENVIRONMENT ON ANATOMICAL STRUCTURE

The summer of 1928 differed from that of 1927 in being uniformly sunny, with rains falling in the right amount and at the right time for maximum sugar production. The increase in sugar, which averaged 2 per cent more than in 1927, was reflected to a certain degree in a changed anatomical structure.

A study of Table 2 shows that, although the weight of the beets fluctuates indifferently in the two seasons, characters such as ring number and width of central core and first ring appear to reflect very definitely seasonal differences. The average ring count is, on the whole, conspicuously and consistently higher in 1928 than in 1927, except for a small number of selections where the ring number did not change. In selection No. 4545-24 (Table 3) the average ring count was 11 for both seasons; selection No. 5656-24 showed a similar conformity, but there was a greater variation in the weight of the beets; selection No. 3956-24 stands out preeminently in both seasons because of its large ring number; selection No. 1161-24 gave a low ring count in both years, but the coefficient of variability was slightly larger in the first year. The ring-density coefficient showed a fair degree of uniformity when individual selections were compared.

Selection No. 1228s-25 had the lowest ring-density coefficient, that is, less than 2 in both years. In selection No. 4545-24 the coefficient reached the high figure of 2.7 and 2.6 for the two seasons. In selection No. 3956-24 the coefficient was also very high, which was quite in harmony with the large ring number.

The size of the first ring and central core showed great seasonal variations, but there was noted a certain degree of uniformity in the individual selections, which seasonal effects were unable to obscure. Selections Nos. 554-24, 3956-24, and 1293s-25 (Table 3) had a small first ring in both seasons. In some of the large-ringed types the diameter of the first ring remained above a mean of 20 mm. in both years, although there was a general tendency toward a smaller ring in the second year. The same tendencies were observed in the central core. The small-core types, selections Nos. 554-24, 730-24, 8146-24, and 6077-24 (Table 3) showed the same small core in both seasons, while most of the large-core types suffered an unequal decrease in size the second year.

The number of vascular bundles per centimeter, with few exceptions, was smaller in 1928 than in 1927. The same tendency was observed in the xylem vessels, although actual counts were not made for all selections. These data are in harmony with the finding that the percentage of sucrose was higher in 1928 than in 1927, although it must be remembered that within the different selections there exists little if any correlation between number of bundles and percentage of sucrose. The fact that, regardless of the smaller number of bundles and xylem vessels, the 1928 beets appeared somewhat more woody than the 1927 crop may be accounted for by the relatively greater abundance of lignified sugar-sheath cells. Although these cells were not found in many selections, those that showed them in 1927 developed them even more abundantly in 1928. In certain selections, notably Nos. 7794-24, 4545-24, and 2445s-25, the lignification in the sugar sheath was very extensive.

RELATION OF STRUCTURE TO PERCENTAGE OF SUCROSE AND TYPE PURITY

In summing up the results of their extensive phytotomic studies on sugar beets, the early investigators conceded that while it was possible to separate rich and poor beets on account of single anatomical differences, it was hopeless to try to distinguish between beets that differed only slightly in their percentage of sucrose; and since even positively classified beets performed in a contradictory fashion the second season, it was concluded that anatomical studies had no practical significance in beet-breeding work. The investigations reported in this paper only emphasize the fact that it would be futile to use a single character as a criterion for percentage of sucrose, especially in dealing with a mixed population. To exemplify: Of four different selections of sugar beets that were recently received from Rocky Ford, Colo., the beets of two of the selections had the same broad vascular zones, and similar narrow first rings and small central cores, but while one sample tested 17 per cent the other tested only 12.5 per cent. Wherein did they differ, and which structural configuration put one type in the poor and the other in the rich class? The beet rich in sugar had 13 rings to the 10 of the low-sucrose beet. The

central core of the rich beet had fewer rays and the vascular rings fewer bundles than the poor beet. A microscopic study showed further that a large part of the sugar-sheath cells of the poor beet were lignified. On the other hand, there appeared to be little difference in the expanse of the phloem tissue and the development of interzonal parenchyma. The larger ring number and the slightly greater ring-density coefficient appeared to be the only features that separated the rich from the poor beet, for the presence of lignified sugar-sheath cells, though suggestive of poor quality, would not have been a safe criterion, since another beet which tested only one-half of 1 per cent higher showed no lignification.

The practical beet grower would probably have relied on the color differences of the flesh to classify the two types, and the numerous observations made in the course of these studies, which have been referred to earlier, give support to the belief that there exists a correlation between flesh color and sugar content. In the present case the flesh of the rich beet was a uniform white, while that of the poor beet was a watery yellow. The beets from the fourth sample were comparatively small and tested very high. They had, of course, a high ring-density coefficient, but the most distinctive character was the prodigious development of the phloem tissue, as can be seen in Figure 10, A and B.

The studies on the different beet selections have shown that the anatomical configuration associated with high or low sugar is not necessarily the same in all selections. Broad vascular zones are of little significance if associated with a small ring number or watery flesh; on the other hand, broad interzonal parenchyma may not necessarily be indicative of poor quality. Undoubtedly, a large ring number, a high ring-density coefficient, broad vascular zones associated with narrow bands of interzonal parenchyma, a fairly large number of evenly spaced bundles with well-developed phloem, a moderate number of xylem vessels, and absence of lignification in the sugar sheath are all indicative of high sugar. The relative development of any of these characters may be different in the different selections, and their influence on the percentage of sucrose in a given selection can be ascertained only through systematic study. Since uniform performance can be safely looked for only in relatively pure types, sugar-beet breeding experiments should take cognizance not only of the factors that express high sugar content but also of those that guarantee type purity. The breeding experiments of recent years have been fruitful in securing types of uniform leaf and root characters, and although at present many of these pure strains are not high producers, there are others that compare favorably with the best commercial stock. The anatomical study of a number of such selections has shown that structural differences exist which in some instances are sufficiently well marked to characterize a selection. In other types well-marked tendencies were observed which seasonal influences partly obscured but did not obliterate.

It is safe to predict that once the different types have become, through continuous inbreeding, sufficiently homozygous to assure uniformity in subsequent generations, it will be possible to give their anatomical type picture, which would more closely delimit them than could be done by external morphological characters alone.

A knowledge of the particular anatomical configuration of each type would not only aid in keeping the type pure, but would expedite the selection process through the elimination of such mother beets as showed distinct anatomical differences. If, for example, there is within a selection a strong tendency toward the development of a small core, the elimination of large-core mother beets should aid in securing more uniform seed, especially where group selection is already practiced.

Additional studies carried on over a number of years must demonstrate which characters in the different selections can be relied on to serve as a criterion for purity of type as well as to determine the actual relation to sugar content. Since climatic factors influence the performance of the crop, and since performance and structure are for physiological reasons interrelated, the effect of a different season must be reflected in a changed structure; yet these influences probably do not have more than a quantitative value and should not obliterate the trustworthy type characters. Other environmental factors which often affect the yield should have little influence in these selection studies, since through proper management their effects should be reduced to a minimum.

The different beet selections are more or less sharply defined morphologically by their leaf and root characters, while structurally they reflect certain peculiarities which are more strongly realized in some selections than in others. The possibility of a causal relationship between gross morphology and inner structure suggests itself only to be answered negatively after a study of Tables 1, 3, and 10.

TABLE 10.—Yield of 30 sugar-beet selections grown at Fort Collins, Colo., 1927

Selection No.	Sucrose	Sugar per acre	Beets per acre	Selection No.	Sucrose	Sugar per acre	Beets per acre
	<i>Per cent</i>	<i>Pounds</i>	<i>Tons</i>		<i>Per cent</i>	<i>Pounds</i>	<i>Tons</i>
150-24.....	15.53	4,584	14.75	1845s-25.....	14.84	4,764	16.05
193R-25.....	15.03	4,186	13.19	2287-24.....	15.14	4,350	14.36
554-24.....	15.24	3,953	12.97	2310s-25.....	14.53	4,936	16.98
600s-25.....	12.73	1,413	18.66	2340s-25.....	14.64	5,675	19.03
730-24.....	14.53	4,815	16.56	2445s-25.....	15.03	4,658	15.48
898-24.....	14.64	5,170	17.66	3403-24.....	13.53	4,396	16.23
992s-25.....	15.24	3,525	11.56	3956-24.....	13.94	4,714	16.80
1161-24.....	14.53	3,800	13.06	4545-24.....	15.03	3,843	12.78
1223s-25.....	14.13	4,299	15.20	4621-22.....	15.14	4,294	14.17
1293s-25.....	14.64	3,824	13.06	5856-24.....	15.05	4,612	15.95
1427-24.....	13.44	4,386	16.23	5942-24.....	15.34	4,838	15.77
1569-24.....	13.13	3,921	14.92	6077-24.....	14.24	3,857	13.53
1591-24.....	15.03	4,714	15.67	7794-24.....	12.84	3,834	14.92
1612-24.....	15.03	4,714	16.14	8146-24.....	13.74	4,801	17.47
1818s-25.....	14.53	5,072	17.28	8753-24.....	14.74	4,815	16.33

The various selections differ widely in their outer appearance and show no definite connection between root and foliage characters. Spreading or erect leaf habit may be associated with large or small leaf area, and this character in turn may have no relation to the number of leaves characteristic of the different selections. It must be admitted at the outset that a relationship between outer appearance and inner structure exists only for individuals of a given selection and that it is unsafe to extend the characterization to other selections. Slender elongated roots may have either a large or a small first ring or central core, or a small or large ring number. Poor selections, characterized

by a small ring number, appeared at first glance to be associated with a comparatively small leaf area; yet the best selections, as judged by the number of rings, also had a small leaf area. The shape of the root shows little consistent relation to the inner structure. In one selection a parsnip-shaped root was associated with a large central core, while in another selection of that type both core and first ring were small.

These results only emphasize the necessity of studying the anatomical configuration of all established and genetically important types in order to know the inherent tendencies, to preserve them, and to extend their usefulness along desired lines.

SUMMARY

An anatomical study of 30 sugar-beet selections, comprising 1,700 individuals, indicates that the structural configuration of the different selections, for certain characters at least, is specific, and suggests the possibility of obtaining for those strains which through continuous selection have become homozygous an anatomical type picture which seasonal effects may alter but not obliterate and which would aid in delimiting a type and keeping it pure. In order to obtain such type pictures it is necessary to examine entire cross sections of as many beets of a selection as can be obtained, although in case of prospective seed beets the measurements must necessarily be limited to a median section of a cork-borer sample through the central neck region.

Most of our cultivated sugar beets have ivory-white flesh but in poor strains it is of a watery greenish or yellowish hue. The sharpness of the zonation as seen in a cross section is bound up with the color of the flesh, being least marked in pure-white individuals and most prominent in poor, watery ones. The relative width of the vascular zones and the interzonal parenchyma varies indifferently, though in some of the selections the vascular tissue forms conspicuously broad rings.

The number of mature rings is about one-half of the total number produced. The latter is consistently and conspicuously high in some selections and relatively low in others, but in most of the selections examined marked differences were not found. A high total ring number is frequently correlated with a high mature number, but occasionally the opposite is true. The relative ring width of the mature rings is usually related to the weight and slightly to the ring number of the beet, but this correlation is absent in beets with a relatively small ring number.

The ring-density coefficient can be considered an index to percentage of sucrose, except where ring number or ring width, for some reason or other, shows no relationship. The size of the central core and first ring is in certain selections uniformly large or small, so that aberrant forms can be easily detected and eliminated. The size of the central core is related neither to the percentage of sucrose nor to the weight of the beet, nor does the number of core rays show any relation to these factors, except that in very large cores the number of rays is apt to be high.

Although vascular-ring prominence and sugar content are in general independent variables, in beets where broad vascular zones are associated with a high ring-density coefficient a high sugar content is

usually assured. The number of vascular bundles of a ring bears no relation to the percentage of sucrose and only a slight relation to beet size, since as a rule a large beet will have fewer bundles per unit distance than a small beet. The number of xylem cells shows an even greater variation and the data are less reliable, since the size of the cells varies greatly. There appears to be no connection between number of xylem cells and percentage of sucrose. A certain number of selections showed the presence of a smaller or larger number of lignified sugar-sheath cells. These selections were hard to section but were not necessarily poor in sugar, although within a selection there was a tendency to develop a larger amount of lignified sheath cells in poor beets than in rich ones. While the phloem tissue is well developed in most beets, very rich individuals always have a very massive phloem.

The size of the interzonal parenchyma cells shows no important correlation with percentage of sucrose, but may be related within a selection to the width of the rings.

In comparing beets grown in the seasons of 1927 and 1928, it was found that those grown in 1928 had in general a higher number of rings, a smaller central core, and a smaller first-ring diameter than those grown in 1927. Since the differences were only of degree, and since the different selections reacted similarly, type characters were not obliterated. In 1928 the beets averaged higher in percentage of sucrose than in 1927. This increase in sugar appears to be reflected structurally in a higher ring number and a relatively smaller central core and first ring.

The anatomical type picture appears to vary in different beet selections, and although the structural features associated with high sugar appear to be understood, the ideal structural configuration is not always the same. Although performance and structure are interrelated phenomena, the study of any one character may not give a correct evaluation of the sugar-storing capacity of a beet, since the same character in a new combination may influence performance in a different way. This may explain why, for example, beets with comparatively narrow vascular zones are not necessarily poor sugar producers.

LITERATURE CITED

- (1) ARTSCHWAGER, E.
1927. MICRO- AND MACROSPOROGENESIS IN SUGAR BEET WITH SPECIAL REFERENCE TO THE PROBLEM OF INCOMPATIBILITY. *Mem. Hort. Soc. New York* 3: 295-297.
- (2) BOLSUNOW, J. J., and ORLOWSKY, N. J.
1927. BEITRÄGE ZUR UNTERSUCHUNG EINER KOLLEKTIONS-AUSSAAT VON 100 RÜBENSORTENMUSTERN. *Ztschr. Pflanzenzucht.* 12: [305]-325, illus.
- (3) DOSTÁL, R.
1928. ÜBER DIE TANGENTIALE POLARITÄT DER BETA-WURZEL ALS FOLGE DER TORSION IHRER ZUWACHSZONEN. *Ztschr. Wiss. Biol., Abt. E* 5: [325]-339, illus.
- (4) GESCHWIND, L.
1901. SUR LES RELATIONS EXISTANT CHEZ LA BETTERAVE ENTRE LA GENÈSE DU SACCHAROSE ET LA STRUCTURE DE LA RACINE. *Bul. Assoc. Chim. Sucri. et Distill.* 18: 785-796, illus.
- (5) HOFFMANN, M.
1903. DIE ZELLE ALS SELEKTIVES MERKMAL IN DER RÜBENZUCHT. *Bl. Zuckerrübenbau* 10: 206-211, illus.

- (6) JANASZ, S.
1904. BESCHREIBUNG EINIGER ZUCKERRÜBENRASSEN. Mitt. Landw. Inst. Breslau 2: [913]-970, illus.
- (7) KRAUS, E.
1903. UNTERSUCHUNGEN ZU DEN PHYSIOLOGISCHEN GRUNDLAGEN DER PFLANZENKULTUR. I. DIE WACHSTUMSWEISE DER BETA-RÜBEN. Naturw. Ztschr. Land u. Forstw. 1: 220-236.
- (8) PACK, D. A.
1927. RING DENSITY OF SUGAR BEETS AS A CHARACTER FOR SELECTION. Amer. Jour. Bot. 14: 238-245, illus.
- (9) PEKLO, J.
1908. HISTOCHEMISCHES ÜBER DIE LOKALISATION DER SACCHAROSE IN DER ZUCKERRÜBE. Österr.-Ungar. Ztschr. Zuckerindus. u. Landw. 37: 153-174, illus.
- (10) SCHINDLER, F., and PROSKOWETZ, E. VON, JR.
1889. ZUR CHARACTERISTIK TYPISCHER ZUCKERRÜBENVARIETATEN. Österr.-Ungar. Ztschr. Zuckerindus. u. Landw. 18: 351-406, illus. (Also in Ztschr. Ver. Deut. Zuckerindus. (n. F. 27) 40: 96-126, illus. 1890.)
- (11) SCHNEIDER, J.
1900-01. ÜBER DIE HISTOLOGIE DER ZUCKERRÜBE. Ztschr. Zuckerindus. Böhmen 25: 305-326, illus.
- (12) SEELIGER, R.
1920. ÜBER DIE RINGDICHTEN ALS AUSLESEMERKMAL BEI DER ZUCKERRÜBE. Mitt. Biol. Reichsanst. Land u. Forstw. 18: 64-68, illus.
- (13) VIVIEN, A.
1920. SACCHAROGÉNIE SÉLECTION DE LA BETTERAVE À SUCRE. Bul. Assoc. Chim. Sucr. et Distill. 38: 143-163.
- (14) VRIES, H. DE
1879. BEITRÄGE ZUR SPECIELLEN PHYSIOLOGIE LANDWIRTSCHAFTLICHER CULTURPFLANZEN. VII. WACHSTUMSGESCHICHTE DER ZUCKERRÜBE. Landw. Jahrb. 8: [417]-498, illus.
- (15) WIESNER, J.
1867. EINLEITUNG IN DIE TECHNISCHE MIKROSKOPIE NEBST MIKROSKOPISCHTECHNISCHEN UNTERSUCHUNGEN. 271 p., illus. Wien.

TOXICITY OF BIKUKULLA FORMOSA (WESTERN BLEEDINGHEART)¹

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INTRODUCTION

Three different native plants of the genus *Bikukulla* (also known as *Dicentra*) have previously been studied in this laboratory and reported on with respect to their poisonous properties and danger to grazing animals.² One of these, *B. cucullaria*, was found to contain an intensely poisonous alkaloid and to be a grave danger to animals on ranges where it may occur. The other two, *B. canadensis* and *B. eximia*, although found to contain alkaloids in appreciable quantity, could be classed as harmless, since their basic contents were not toxic. Still another member of this group of plants, *B. formosa*, has been found growing wild in the West and is the subject of the present paper.

BOTANICAL DESCRIPTION OF BIKUKULLA FORMOSA

Bikukulla formosa (Andr.) Coville. Western bleedingheart. (Fig. 1.) A smooth, succulent perennial with thick fleshy branching rootstock; stemless; flowering stalks several, 6 inches to 2 feet high; flower clusters twice branched, slightly taller than the leaves; leaves twice ternately dissected, long-stemmed; corolla flattened, ovate-cordate at the base, the four petals 7 to 9 lines long, united up to the middle, withering persistent about the seed pod, crests of inner petals tubular, conspicuous; sepals two, scalelike; stamens in two sets of three each; stigma one, 2-lobed; seed pod 1-celled.

Shady woods of British Columbia (where first discovered by Menzies), south through the lower mountain woods of western Washington and Oregon, the coast ranges of California to Alameda County, and in the Sierra Nevada to Tulare County. More at home in the moist climate from British Columbia to Oregon.

CHEMICAL EXAMINATION

Although most of these plants have been the subject of more or less chemical study, little is known concerning the physiological properties of their constituents. Apparently all of them contain alkaloids, but in few instances have these been investigated physiologically.

The alkaloids of *Bikukulla formosa* were studied by Heyl,³ who, working with roots of plants obtained from one of the German botanical gardens, was able to isolate protopine and two other crystalline alkaloids. Protopine is a well-known alkaloid found among the numerous constituents of opium and also in a variety of plants.

¹ Received for publication Dec. 31, 1929; issued May, 1930.

² BLACK, O. F., EGGLESTON, W. W., KELLY, J. W., and TURNER, H. C. POISONOUS PROPERTIES OF BIKUKULLA CUCULLARIA (DUTCHMAN'S-BREECHES) AND B. CANADENSIS (SQUIRREL-CORN). Jour. Agr. Research 23: 69-78, illus. 1923.

EGGLESTON, W. W., BLACK, O. F., and KELLY, J. W. A BOTANICAL AND CHEMICAL STUDY OF BIKUKULLA EXIMIA, WITH A KEY TO NORTH AMERICAN SPECIES OF BIKUKULLA. Jour. Agr. Research 39: 477-481, illus. 1929.

³ HEYL, G. UEBER DIE ALKALOIDE VON DICENTRA FORMOSA (ANDR.) D. C. Arch. Pharm. 241: 313-320. 1903.

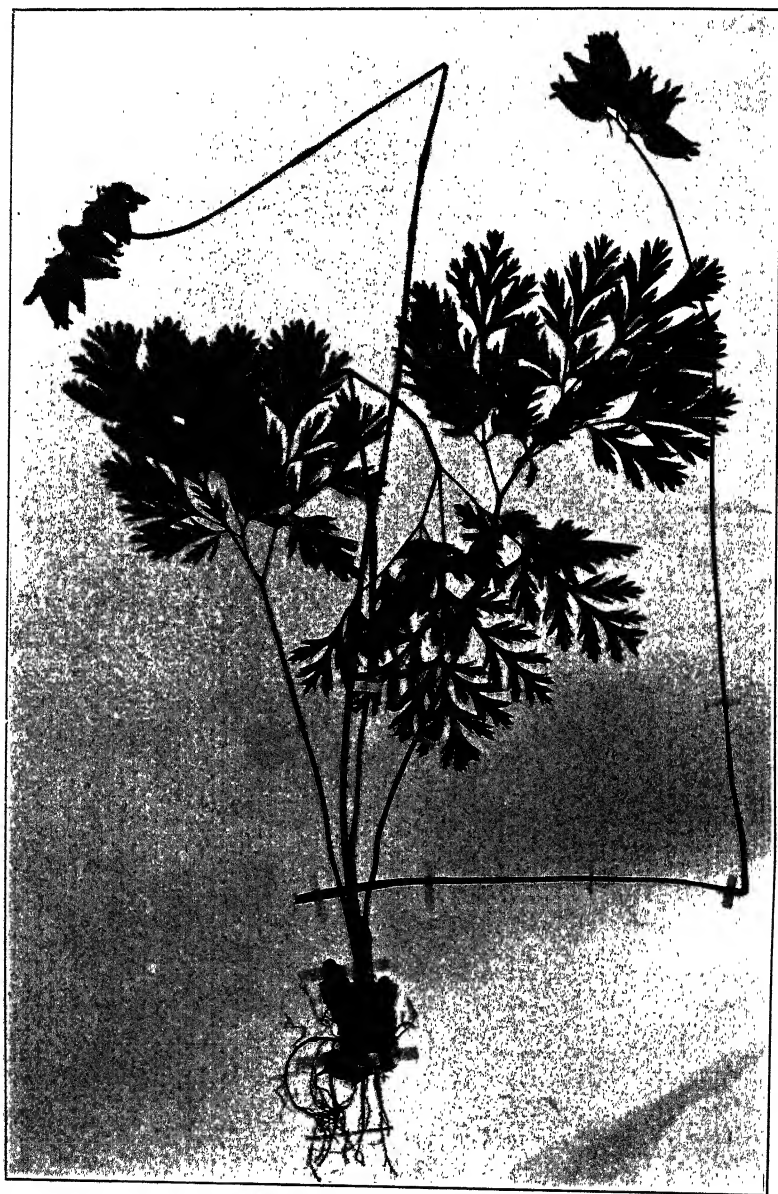


FIGURE 1.—Flowering plant of *Bikukulla formosa*

It is physiologically active, producing local anesthesia and, in mammals, narcosis and convulsions, but it has no extensive use in medicine. The other two alkaloids were not named by Heyl, and nothing is known concerning their physiological properties.

In June, 1928, a quantity of *Bikukulla formosa* was collected in the Cascade Range about Fish Lake ranger station, Santiam National Forest, Oreg. The tops were dried and sent to Washington, D. C., where they arrived in fair condition. Eight hundred grams of this material, ground to a moderate fineness, was packed in a percolator and moistened with 80 per cent alcohol weakly acidified with acetic acid. After standing 24 hours it was exhaustively percolated with alcohol of the same strength, and the solution thus obtained was concentrated by vacuum distillation to about 500 c. c. A moderate quantity of 10 per cent lead acetate solution was added, the precipitate filtered, and the excess of lead removed by dilute H_2SO_4 . The resulting solution was partially neutralized with solid $CaCO_3$, then concentrated to a volume of about 300 c. c. This extract was made strongly alkaline with Na_2CO_3 , resulting in a voluminous precipitate, which was removed by filtration and the filtrate shaken out twice with ether. Further treatment with ether and other solvents failed to remove any further basic material, showing that the extraction had been complete.

The precipitated bases and residues from the ether extractions, which dried to a brown sirupy material, were united and treated with hot benzol, which left a slight quantity of black tarry substance undissolved. The crude basic material prepared by this procedure weighed 8.3 gm., or just over 1 per cent of the plant material. Heyl⁴ stated that he obtained 3 per cent of crude alkaloid, but as he worked with the roots alone, the difference between his results and the writers' may well have been due to the presence of a much greater percentage of alkaloid in the roots than in the tops.

The crude basic material, when freed from the solvent and dried, consisted of a light-brown amorphous gummy mass which grew darker in color on standing in the desiccator and responded to all the ordinary tests for alkaloids. It showed no tendency to crystallize, although Heyl reports that under the same conditions his material yielded crystals of protopine. The crude preparation described was subjected to a number of methods of purification, but neither through these nor by the use of any of the ordinary solvents or combinations of such solvents could any well-defined separation or crystallization be brought about.

PHYSIOLOGICAL TEST OF THE EXTRACT

Since the main object of this investigation was to gain a practical knowledge of the toxicity of the plant to animals, the crude basic material afforded a starting point. Two-tenths of a gram was dissolved in very dilute hydrochloric acid and the volume made up to 5 c. c. A white mouse weighing 21 gm. was given 0.25 c. c. of this solution by subcutaneous injection. The animal remained in a quiescent, semistupified condition for 15 minutes and was then seized with convulsions which lasted intermittently for 30 minutes,

⁴ Heyl, G. Op. cit.

when it died. The lungs were collapsed, the extremities blue, the heart distended, and death was probably due to respiratory paralysis. By gradually diluting the solution, progressively weaker doses of the base were administered to mice in the same way until a dilution was reached that failed to prove fatal. In all cases the symptoms of poisoning were the same.

From this procedure it was determined that the lethal dose of the crude alkaloids lies between 5 and 2.5 mgm. for a mouse of about 20 gm. in weight; or, calculated on the basis of percentage of alkaloids in the plant, it would be represented by from one-half to one-fourth gram of dry plant.

Although the toxicity of the plant to mice does not prove that it is equally poisonous to other animals, it is evident that *Bikukulla formosa* should be placed in the category of poisonous plants and should be regarded as a potential danger to livestock wherever it is found.

FERRIC CITRATE AS AN INGREDIENT OF MINERAL MIXTURES IN PAIRED-FEEDING EXPERIMENTS WITH GROWING SWINE¹

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INTRODUCTION

During the summer of 1927 there was undertaken at the Illinois Agricultural Experiment Station a critical study of the ingredients of mineral mixtures in the ration of growing swine. The methods adopted for use in this study differ fundamentally from those employed in previous experimental work on the question. The feed intake of each pig was under control throughout the experiment, as all pigs were fed in individual feeding crates. The pigs were fed in pairs, so that for each pig receiving the supplement under investigation there was a strictly comparable pig which resembled it closely at the start of the experiment and consumed the same amount of feed throughout. At the present time the work is being confined to a study of definite chemical compounds superimposed singly upon an otherwise well-balanced, practical ration, not particularly rich in the mineral elements contained in the supplement used.

Because of its frequent use in mineral mixtures, ferrous sulphate (copperas) was the first compound to receive attention. Iron is known to be required in animal nutrition, although most farm feeds contain this element in extremely small concentration. The results of this first test, as well as a discussion of the advantages of the paired method of feeding, have already been published.³

METHODS

On account of the negative nature of the results of feeding additional iron in the form of copperas it was decided to test another compound of iron, the thought being that possibly a ferrous salt would not be as suitable as a ferric salt. Ferric citrate was the compound selected to be fed in the second test. The methods of procedure were those outlined and used in the preceding test. Briefly, they consisted of feeding both pigs of each pair the same amount of a well-balanced ration, containing no feed conspicuously high in iron. One pig received only the basal ration, while the other received the same amount of the basal ration and in addition 3 gm. daily of ferric citrate in aqueous solution.

The pigs were paired on a basis of weight, sex, blood lines, thrift, vigor, type, and prospective outcome. Ten pairs of pigs were fed. They were all pure-bred Duroc-Jerseys raised on the University farm. Their weights varied from 64 to 90 pounds at the beginning of the

¹ Received for publication Dec. 3, 1929; issued May, 1930.

² Grateful acknowledgment is made to F. J. McClure for the results on the iron and red-cell content of the blood.

³ CARROLL, W. E., and MITCHELL, H. H. RESULTS OF FEEDING COPPERAS IN PAIRED-FEEDING EXPERIMENTS WITH GROWING SWINE. *Amer. Soc. Anim. Prod. Proc.* 1927: 73-82. 1928. (See also STUDY IN-
GREDIENTS IN COMPLEX MINERAL MIXTURES. *Ill. Agr. Expt. Sta. Ann. Rpt.* 41: 132-136. 1928.)

test, though no two pigs of a pair varied initially more than 5 pounds in weight and most of them weighed within 2 or 3 pounds of each other.

The basal ration used was the low-iron ration of the previous test and consisted of yellow corn, soybean-oil meal, linseed meal, alfalfa meal, calcium carbonate, and sodium chloride. The proportion of the ingredients was changed as the pigs increased in weight. The changes made are indicated in Table 1.

TABLE 1.—*Variations in the proportion of the ingredients of the ration fed the growing swine at different live weights*

Feeds	Proportion in which the feeds were mixed for pigs weighing—		
	100 pounds or less	100 to 150 pounds	Over 150 pounds
Ground corn.....	70	80	90
Soybean-oil meal.....	18	12	6
Linseed meal.....	6	4	2
Alfalfa meal.....	6	4	2
Calcium carbonate.....	1	1	1
Sodium chloride.....	0.5	0.5	0.5

The chemical composition of the feeds used throughout the experiment, determined upon composite samples, is given in Table 2.

TABLE 2.—*Percentage chemical composition of the feeds on the fresh basis*

Feeds	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract	Iron
Soybean-oil meal.....	88.56	42.54	5.60	6.04	5.84	28.54	0.0291
Corn.....	88.64	8.27	3.39	1.38	2.43	71.17	.0085
Linseed meal.....	89.95	34.44	5.05	5.39	8.91	36.16	.0153
Alfalfa meal.....	88.98	15.31	2.08	7.13	27.29	37.22	.0249

The pigs were fed twice daily in individual feeding crates. The mixed ration was weighed to one-tenth of a pound and fed in one end of a divided metal trough with which each crate was equipped. Water was available in the other end of the trough. The feeds were given dry except that the solution of ferric citrate was poured over the ration of the test pigs just before the pigs were turned into their crates. The pigs were watched closely in an attempt to keep the feed consumption up to the limit of the appetite of the lightest feeding pig of each pair.

Except while feeding, the pigs were kept on concrete floors, the check pigs being kept in one group and the pigs receiving iron in another. Each group was given a pen in the northwest side of a central swine barn adjacent to which was an outside concrete runway.

The pigs were continued on feed until one or both pigs of a pair reached a final weight of 175 pounds.

RESULTS

The results of this test are summarized briefly in Table 3, which gives for each pig the initial and final weights;⁴ the weekly, the total,

⁴ Initial and final weights are averages of weights on three consecutive days.

and the average daily gains; as well as the total feed eaten, the average daily ration, and the feed eaten per pound of gain.

TABLE 3.—Weights, gains, and feed consumption of 10 pairs of pigs, one of each pair being on the basal ration alone and the other getting a ferric citrate supplement

	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5	
	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate
Final weight.....	Pounds 175	Pounds 170	Pounds 175	Pounds 159	Pounds 181	Pounds 182	Pounds 174	Pounds 164	Pounds 181	Pounds 175
Initial weight.....	86	82	80	75	70	72	67	64	80	88
Total gain.....	89	88	95	84	111	110	107	100	91	87
Period on test (days)	84	84	98	98	106	106	98	98	85	85
Average daily gain..	1.06	1.05	0.97	0.86	1.05	1.04	1.09	1.02	1.07	1.02
Gain or loss in weight:										
Week 1.....	0	-2	0	-3	3	-2	1	-2	0	-2
Week 2.....	5	6	5	6	6	4	6	7	6	7
Week 3.....	5	6	6	3	3	5	5	5	4	3
Week 4.....	6	8	9	8	8	8	8	6	7	6
Week 5.....	9	7	5	10	8	10	9	8	9	10
Week 6.....	6	6	9	7	6	9	9	10	9	9
Week 7.....	10	11	10	7	8	6	8	9	9	6
Week 8.....	8	6	7	8	7	7	9	8	7	9
Week 9.....	10	13	12	11	11	10	9	7	12	10
Week 10.....	11	6	10	9	3	2	3	5	9	8
Week 11.....	8	9	7	9	10	10	11	9	6	12
Week 12.....	11	12	9	5	9	9	7	11	^a 13	^a 9
Week 13.....	-----	-----	-1	0	8	10	9	8	-----	-----
Week 14.....	-----	-----	7	4	8	9	13	9	-----	-----
Week 15.....	-----	-----	-----	-----	^a 13	^a 13	-----	-----	-----	-----
Total feed eaten.....	315	315	351	351	412	412	367	367	323	323
Average daily ration..	3.74	3.74	3.58	3.58	3.88	3.88	3.74	3.74	3.80	3.80
Feed per pound of gain.....	3.53	3.58	3.69	4.18	3.71	3.74	3.43	3.67	3.55	3.71

	Pair 6		Pair 7		Pair 8		Pair 9		Pair 10	
	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate
Final weight.....	Pounds 172	Pounds 174	Pounds 174	Pounds 169	Pounds 188	Pounds 178	Pounds 176	Pounds 180	Pounds 176	Pounds 167
Initial weight.....	83	87	78	82	78	74	72	71	69	66
Total gain.....	89	87	96	87	110	104	104	109	107	101
Period on test (days)	84	84	91	91	113	113	120	120	126	126
Average daily gain..	1.06	1.04	1.05	0.96	0.97	0.92	0.87	0.91	0.85	0.80
Gain or loss in weight:										
Week 1.....	1	0	-2	1	-1	-2	0	-1	1	-1
Week 2.....	6	6	3	1	7	8	6	5	5	8
Week 3.....	8	4	5	3	2	1	1	1	5	2
Week 4.....	4	6	7	9	9	7	6	5	7	7
Week 5.....	7	9	10	7	8	6	7	9	8	6
Week 6.....	9	6	6	6	6	6	9	8	-1	-1
Week 7.....	8	9	10	9	6	8	9	8	5	6
Week 8.....	8	7	6	4	9	6	6	7	6	5
Week 9.....	12	14	12	10	10	11	10	10	7	7
Week 10.....	7	7	12	8	3	3	8	9	5	8
Week 11.....	9	8	7	11	11	10	-2	4	7	8
Week 12.....	10	11	11	15	6	10	2	3	6	3
Week 13.....	-----	-----	9	3	11	9	6	5	3	4
Week 14.....	-----	-----	-----	-----	4	5	6	6	5	6
Week 15.....	-----	-----	-----	-----	9	10	8	7	9	13
Week 16.....	-----	-----	-----	-----	^a 10	^a 6	11	10	11	9
Week 17.....	-----	-----	-----	-----	-----	-----	^a 11	^a 13	10	8
Week 18.....	-----	-----	-----	-----	-----	-----	-----	-----	8	3
Total feed eaten.....	317	317	349	349	421	421	417	417	398	398
Average daily ration..	3.78	3.78	3.84	3.84	3.73	3.73	3.48	3.48	3.16	3.16
Feed per pound of gain.....	3.56	3.65	3.64	4.01	3.83	4.05	4.01	3.83	3.72	3.94

^a 8 days in this period. Initial and final weights are the averages of 3 weights taken on consecutive days. Single weights were used in the other cases.

It is a matter of no surprise that the rates of gain are less than the ration is capable of supporting under conditions of unrestricted feeding. Obviously the prime requisite in a feeding experiment such as this is to obtain closely comparable controls. Submaximal feeding and gaining are necessary consequences of proper experimental control and do not detract from the significance of the comparisons made, since a ration deficient in some particular constituent for growth should be improved in its growth-promoting properties at all levels of feeding by the addition of the proper supplement.

As would be expected, there was considerable variation in both rate and economy of gain among the various pairs. The difference between pigs of the same pair, however, was not great. The largest difference in both rate and economy of gain existed between the pigs of pair 2, a difference of 12.1 per cent in rate and 12.5 per cent in economy of gain.

The check pig in every case, except pair 9, made more rapid and economical total gains than the test pig that ate the same amount of feed and in addition received 3 gm. of ferric citrate daily throughout the test. The results suggest, as did the copperas results of the preceding test with reference to a tankage ration, that added iron salts may depress the rate of gain of growing pigs. However, a further analysis of the data by statistical methods indicates that only an inconsiderable probability has been established for this interpretation.

The 10 pairs of pigs were on experiment for from 12 to 18 weeks. By considering each week for each pair an experimental datum, there are 143 pair weeks, affording as many comparisons of test and control pigs. If the iron supplement were without value in promoting growth and without harmful effect in retarding growth, the chances are even that in any one week on equal intakes of food the gain in weight of the pig receiving iron will exceed that of its control mate, or will be less than that of its pair mate. With 143 comparisons, therefore, the ideal result, if the iron citrate supplement were without effect, would be 71.5 favoring the check pig and 71.5 favoring the test pig. If the weekly gains in Table 3 are consulted, it will be found that 79.5 comparisons favor the check pig, and 63.5 favor the iron-fed pig. The ideal result of 71.5 would of course be a very unlikely outcome in a negative experiment. The question at issue is whether the deviation from this ideal obtained in this particular experiment, i. e., $79.5 - 71.5 = 8$, could reasonably result from the operation of chance, which would have determined it if the iron supplement were without effect on growth. This is a proposition in which the assessment of probabilities may be made by means of the binomial distribution.⁵ The standard deviation of the frequency distribution of the outcome of 143 events, each of which may result with equal probability in either of two ways, is given by the expression $\sqrt{0.5 \times 0.5 \times 143}$, which is equal to 5.98. The deviation of 8 from the ideal outcome is only 1.34 times this standard deviation. It would occur by chance about once in six trials, and hence its occurrence in any one trial is not indicative of the operation of any but chance factors in the determination of the outcome of the weekly comparisons of the gains of pair mates.

⁵ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., p. 63. Edinburgh and London. 1928.

THE COMPOSITION OF THE BLOOD

The iron content and the concentration of erythrocytes were determined upon the blood of 7 of the 10 pairs of pigs, the blood samples being taken from the tail at the conclusion of the feeding experiment. Iron was determined by the Kennedy method.⁶ The results will be found in Table 4.

TABLE 4.—*Iron and erythrocyte contents of the blood of the pigs in 7 of the 10 pairs at the conclusion of the feeding test*

ERYTHROCYTES IN MILLIONS PER CUBIC MILLIMETER							
Pig	Pair 2	Pair 3	Pair 4	Pair 7	Pair 8	Pair 9	Pair 10
Control.....	6.40	9.14	6.01	7.47	6.43	6.87	8.12
Ferric citrate.....	7.64	9.25	8.29	8.04	7.49	7.23	8.58

PER CENT OF IRON							
Control.....	0.0455	0.0504	0.0436	0.0406	0.0450	0.0400	0.0414
Ferric citrate.....	.0478	.0556	.0480	.0424	.0455	.0484	.0479

For each of the seven pairs, the pig receiving ferric citrate was found to have blood containing a greater concentration of red blood cells and a greater concentration of iron than its control mate. A consistent outcome of seven such comparisons as these would, according to the binomial distribution

$$\left(\frac{1}{2} + \frac{1}{2}\right)^7$$

occur by chance only twice in 128 trials, and a consistent positive effect, considering the probability of a depressing effect of iron to be nil, only once in 128 trials. Hence, the observations are highly indicative of an effect of supplemental iron on the hemoglobin and red cell content of the blood.

On an average, the blood of the pigs given ferric citrate contained 8,070,000 red cells per cubic millimeter as compared with 7,210,000 for the control pigs, a difference of 11.9 per cent. The average iron content of the blood was 0.0479 per cent for the pigs given ferric citrate and 0.0438 per cent for the control pigs, a difference of 9.4 per cent. The pigs receiving no iron supplement were not anemic,⁷ so that the slightly higher cell count and iron (hemoglobin) content of the blood in all probability induced by the daily administration of 3 gm. of ferric citrate, although of scientific interest, should be accorded no considerable practical importance. In this connection it may be recalled that Whipple has maintained dogs in an anemic condition at one-third the normal hemoglobin level for four years in perfect health and activity.⁸

⁶ KENNEDY, R. P. THE QUANTITATIVE DETERMINATION OF IRON IN TISSUES. *Jour. Biol. Chem.* 74: 385-391, illus. 1927.

⁷ For example, the red-cell count of the pig is given as 6,200,000 per cubic millimeter in the following publication: MARLOFF, R. DIE FRÜHEREN ZÄHLUNGEN DER ERYTHROCYTEN IM BLUTE VERSCHIEDENER TIERE SIND TEILWEISE MIT GROSSEN FEHLERN BEHAFTET. *Pflüger's Arch. Physiol.* 175: 355-370. 1919. The red-cell count is given in the following as 6,215,000 red cells per cubic millimeter as the average obtained from 25 pigs weighing about 100 pounds: PALMER, C. C. MORPHOLOGY OF NORMAL PIGS' BLOOD. *Jour. Agr. Research* 9: 131-140. 1917.

⁸ WHIPPLE, G. H. EXPERIMENTAL ANEMIAS, DIET FACTORS AND RELATED PATHOLOGIC CHANGES OF HUMAN ANEMIAS. *Jour. Amer. Med. Assoc.* 91: 863-867, illus. 1928.

SUMMARY

The administration of ferric citrate at the rate of 3 gm. daily to young growing pigs on a ration of yellow corn, soybean oil meal, linseed meal, alfalfa meal, calcium carbonate, and sodium chloride had no effect upon the rate or economy of gains in 12 to 18 weeks of feeding. The paired-feeding method was used in this trial, involving 10 pairs of pigs.

In seven pairs of pigs, the blood was examined at the conclusion of the feeding experiment. Among these pairs, the pig receiving iron consistently showed a higher red-cell count and a higher iron content of the blood. These results are statistically significant. Their practical importance, however, is inconsiderable, since the pigs not receiving iron were not anemic. There is no reason to believe that the iron supplement conferred any actual benefits upon the pigs receiving it.

THE PRODUCTION AND CURE OF NUTRITIONAL ANEMIA IN SUCKLING PIGS¹

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INTRODUCTION

Although the investigation described in the preceding paper (2),² as well as contemporary work in this country (1), has not shown that good rations for young growing pigs are commonly deficient in iron even though they contain no feeds conspicuously high in this element, or that iron supplements to such rations will perform any useful purpose, the case of the suckling pig, subsisting largely or entirely upon the milk of the dam, may very well be in a different category. Although no record in the recent literature has been found of an iron analysis of the milk of the pig, the milk of cattle and of goats (5) contains only 0.0002 to 0.0003 per cent of iron, and human milk seems to contain but slightly more (3). As compared with other feeds (17), these milks, and presumably the milk of the pig also, are extremely poor in iron. Indeed, it has been found possible to induce severe nutritional anemia in rats (19), rabbits (8), and chickens³ by the feeding of cow's milk. The probability of the occurrence of anemia in suckling pigs is thus not remote.

WORK OF OTHER INVESTIGATORS

The actual occurrence of anemia under farm and experimental conditions, and the symptoms by which it is accompanied, have been described by McGowan and his associates (10, 11) in England, and by Doyle, Mathews, and Whiting (4) in this country. Its etiology, however, has been in doubt. McGowan claims that it is due to the feeding of iron-deficient rations to the sow, but Doyle and his associates were not able to confirm this belief. The latter investigators found that some factor in outdoor conditions tended to prevent anemia in suckling pigs. In a later communication (13) it was shown that sunlight was effective in preventing anemia, but that ultra-violet irradiation indoors was ineffective.

The remarkable finding of Hart and his associates (9), observed independently and less directly by McHargue and his coworkers (12), that the administration of copper salts to rats subsisting upon a milk diet prevented the appearance of the anemia that otherwise invariably occurred, afforded the first definite indication for the prevention and cure of nutritional anemia in suckling pigs. Hart himself was the first, in so far as the authors are aware, to apply this experimental finding to young pigs. In a talk given before the thirty-second annual meeting of the United States Livestock Sanitary Association in Chicago in December, 1928, Hart described his work on iron in nutrition,

¹ Received for publication Dec. 3, 1929; issued May, 1930.

² Reference is made by number (italic) to "Literature cited," p. 938.

³ University of Illinois unpublished data.

and presented briefly the results of copper and iron medication of part of one litter of anemic pigs. The treatment was distinctly successful, but for some reason these results have not been included in the report of the paper appearing in the printed proceedings of the society.⁴

EXPERIMENTAL DATA

In a continuation of the work on the value of iron supplements in swine nutrition advantage was taken of this discovery of the function of copper in blood formation. Some preliminary observations on pigs farrowed from sows subsisting upon iron-poor rations (14) had not confirmed the belief of McGowan that anemia was a necessary sequel of such treatment. However, in the following year (spring of 1929) four Duroc-Jersey pigs from a litter of 13, raised under the usual routine at the swine barns, exhibited symptoms similar to those described by McGowan. Accordingly, samples of blood were taken from the tails of these pigs and analyzed for hemoglobin by the acid hematin method of Newcomer (16), the Newcomer plate being standardized by the Van Slyke oxygen capacity method (18).⁵ The pigs were 11 days old, and the hemoglobin contents, expressed in grams per 100 c. c., was 5.05, 5.05, 5.57, and 5.05. Two of the pigs were then given daily by pipette a solution of copper sulphate containing 5 mgm. of copper daily and at the end of a week solutions of both ferric citrate and copper sulphate in amounts equivalent to 25 mgm. of iron and 5 mgm. of copper. One of these pigs died in about a week, but the hemoglobin in its blood had risen from 5.05 to 7.14 gm. per 100 c. c. The hemoglobin of the other treated pig remained at a low level for three weeks and then rapidly increased in the following three weeks to 10.1 gm. per 100 c. c., after which a slight drop occurred. The two untreated pigs showed a decrease in hemoglobin. At 25 days of age, one of them gave a sample of blood containing only 2.85 gm. of hemoglobin per 100 c. c. The iron and copper medication was started at this time, and the animal responded rapidly, so that at an age of 53 days the blood contained 9.62 gm. of hemoglobin per 100 c. c. The second untreated pig was kept in a distinctly anemic condition, with the hemoglobin varying from 2.5 to 3 gm. per 100 c. c., from the twenty-fifth to the sixtieth day of age. It was then given the usual daily dose of ferric citrate and copper sulphate. From a level of 2.96 gm. the hemoglobin increased in three weeks to 8 gm per 100 c. c. These pigs were all kept indoors.

This preliminary experiment confirmed in every way the experiment reported orally by Hart.

Observations were then made upon three litters of pigs that were cared for in the normal manner, no metallic salts being given. The only difference in the treatment accorded the litters was the time from birth at which they were removed outdoors into cindered pens. The first litter, 9 PC, was a vigorous one of four Poland China pigs farrowed on February 25, to which one Duroc-Jersey pig was later added. This litter was removed outdoors to a cindered pen on March

⁴ A popular bulletin describing these and other results of a similar nature on suckling pigs has since appeared (7).

⁵ Five standardizations with pig blood of the Newcomer colored glass disk gave an average of 0.003085 as the disk factor instead of 0.0035 as furnished by the manufacturers of the disk. All acid hematin solutions were prepared in the manner described by Newcomer and all were allowed to stand overnight at room temperature before readings were made. Turbidities, such as those encountered when acid hematin solutions are prepared from avian blood by the Newcomer procedure, were not encountered with pig blood.

15. Very soon after this one of the pigs, 9-30-B, escaped from the pen. Litter 4 PC was also a normal healthy litter of Poland China pigs, which on account of the prevailing bad weather was not removed to the outside until April 11, when the pigs were over 3 weeks of age. Litter 3 DJ was a small litter of Duroc-Jersey pigs which, when 2 weeks of age, appeared to be anemic. Blood samples were therefore taken at this time and for three succeeding weeks. On March 30 this litter was removed to an outside pen. The observations on the blood, including hemoglobin content, and red-cell counts on the first litter, are summarized in Tables 1, 2, and 3. In this and all subsequent experiments, blood samples were taken from an ear vein.

TABLE 1.—*Hemoglobin content and erythrocyte count of the blood of a vigorous litter of pigs*

[Litter 9 PC; ^a farrowed Feb. 25 and moved outside Mar. 15, no medication]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date of observation	Age of pigs	Pig No.				
		9-9-S	9-90-S	9-30-B	9-3-S	2-9-S
1929						
Mar. 6	1 week 2 days	6.37	8.03			6.78
Mar. 13	2 weeks 2 days	6.64	7.86	7.80		7.01
Mar. 20	3 weeks 2 days	8.39	10.68	11.99		9.53
Mar. 27	4 weeks 2 days	9.87	11.11		10.90	12.21
Apr. 3	5 weeks 2 days	11.03	9.74		10.74	10.33
Apr. 10	6 weeks 2 days	11.47	10.56		12.91	12.84
Apr. 17	7 weeks 2 days	11.45	11.90		12.45	10.37

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

Mar. 6	1 week 2 days	5.06	5.52	-----	-----	6.26
Mar. 13	2 weeks 2 days	5.54	5.15	5.16	-----	5.39
Mar. 20	3 weeks 2 days	5.42	6.63	7.65	-----	7.34
Mar. 27	4 weeks 2 days	7.09	7.78	-----	11.70	10.12
Apr. 3	5 weeks 2 days	8.53	7.85	-----	8.53	7.09
Apr. 10	6 weeks 2 days	8.35	9.23	-----	8.53	9.65
Apr. 17	7 weeks 2 days	8.47	7.57	-----	9.44	-----

^a Blood of sow 9 PC contained 9.65 gm. hemoglobin per 100 c. c. when tested Apr. 3.

TABLE 2.—*Hemoglobin in grams per 100 c. c. of the blood of a normal litter of pigs*

[Litter 4 PC; kept inside till Apr. 11; no medication]

Date	Age of pigs	Pig No.			
		4-30-B	4-30-S	4-3-S	4-90-S
1929					
Mar. 20	2 hours	9.31	9.05	11.25	
Mar. 27	1 week	4.00	4.05	(a)	5.40
Apr. 3	2 weeks	3.20	3.69		3.63
Apr. 10	3 weeks	2.55	6.82		3.83
Apr. 17	4 weeks	(b)	9.53		6.82

^a Killed by sow.

^b Died.

TABLE 3.—*Hemoglobin in grams per 100 c. c. of the blood of a small, apparently anemic, litter of pigs*

[Litter 3 DJ; * farrowed Mar. 13; apparently anemic when study started on Mar. 27; sow and litter placed in outside pens Mar. 30; no medication]

Date	Age of pigs	Pig No.		
		3-30-S	3-30-B	3-89-B
1929				
Mar. 27.....	2 weeks 1 day.....	3.34	3.42	-----
Apr. 3.....	3 weeks 1 day.....	2.33	2.26	2.47
Apr. 10.....	4 weeks 1 day.....	9.36	7.40	8.95
Apr. 17.....	5 weeks 1 day.....	12.95	8.16	10.64

* Blood of sow 56 DJ contained 10.30 gm. hemoglobin per 100 c. c. when tested Apr. 3.

The main significance of these observations relates to the anemic condition developing in all pigs, no matter how healthy and vigorous they appeared to be, during indoor confinement, and the rapid alleviation of this condition upon removal to an outdoor cindered pen. According to the results obtained with litter 4 PC (Table 2), from which blood samples were taken two hours after birth, the pig is born with a fairly high level of hemoglobin, which is rapidly reduced during the first week of life.

The most striking illustration of the rapid decrease in the hemoglobin of pigs during the first few days after birth and of the rapid recovery that is effected by the change from indoor to outdoor conditions was obtained with litters 90 DJ and 93 DJ, farrowed from sisters bred to the same boar. These results are summarized in Tables 4 and 5. The hemoglobin measurements are presented graphically in Figure 1 A and B.

TABLE 4.—*Blood hemoglobin and erythrocyte count, and weights, of a litter of pigs kept outdoors*

[Litter 90 DJ; * sow and pigs moved outdoors in a cindered pen on the sixth day after farrowing; no medication]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date	Age of pigs	Pig No.					Average
		90-3-B	90-3-S	90-30-S	90-9-S	90-99-S	
1929							
Apr. 5	6 to 10 hours.....	13.75	11.94	9.68	11.25	11.00	11.52
8	3 days.....	9.05	6.74	8.43	8.16	8.09	8.06
10	5 days.....	7.56	6.90	7.37	6.56	8.27	7.33
17	1 week 5 days.....	5.45	4.64	6.53	4.93	6.94	5.70
24	2 weeks 5 days.....	9.06	5.79	8.30	6.77	8.83	7.75
May 1	3 weeks 5 days.....	8.81	7.11	12.06	8.67	11.47	9.62
8	4 weeks 5 days.....	10.56	11.33	12.44	10.37	12.04	11.35
15	5 weeks 5 days.....	12.64	12.19	10.44	11.33	11.58	11.64
22	6 weeks 5 days.....	12.99	11.65	9.41	10.96	10.79	11.16

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

Date	Age of pigs	Pig No.					Average
		90-3-B	90-3-S	90-30-S	90-9-S	90-99-S	
Apr. 5	6 to 10 hours.....	6.36	6.40	5.87	6.07	-----	6.17
8	3 days.....	5.37	3.84	4.66	3.81	6.18	4.77
24	2 weeks 5 days.....	-----	3.63	5.41	4.48	6.15	4.92
May 22	6 weeks 5 days.....	9.21	9.77	-----	-----	7.29	8.75

WEIGHTS OF PIGS (POUNDS)

Date	Age of pigs	Pig No.					Average
		90-3-B	90-3-S	90-30-S	90-9-S	90-99-S	
Apr. 5	At birth.....	3.0	3.2	2.8	2.6	3.0	2.92
May 22	6 weeks 5 days.....	30.5	31.5	-----	28.0	21.7	27.94

* Blood of sow 90 DJ contained 9.33 and 9.76 gm. hemoglobin per 100 c. c. when tested on Apr. 3 and Apr. 10, respectively.

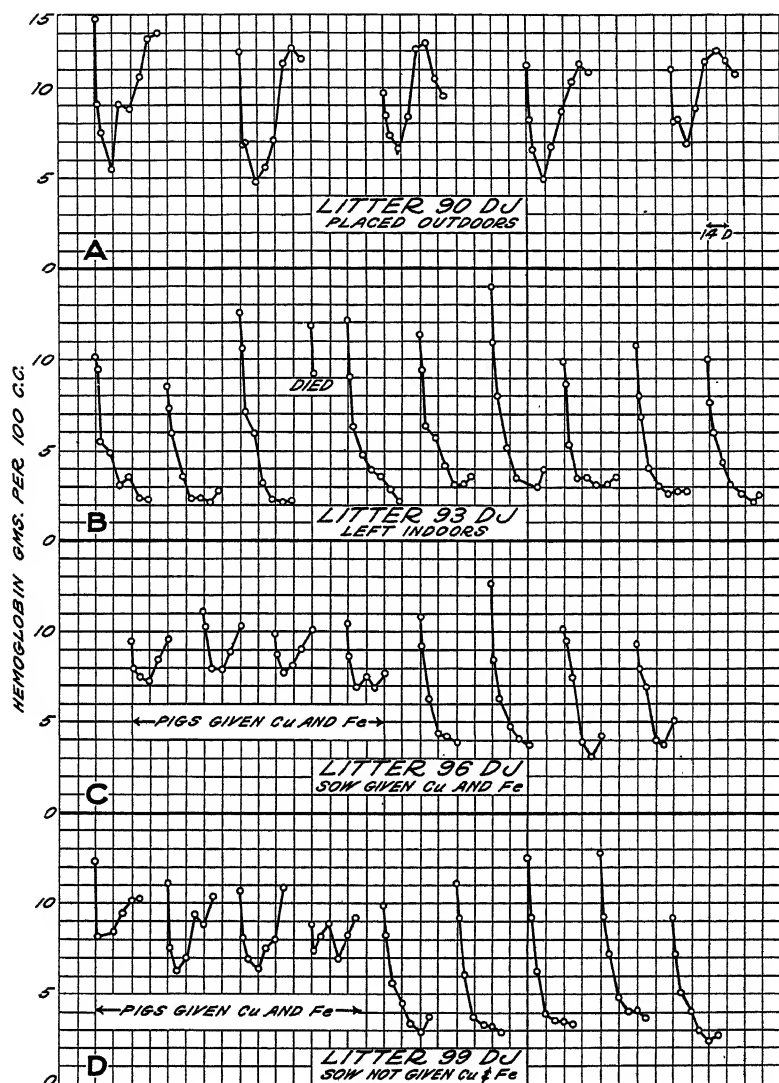


FIGURE 1.—The effect on the concentration of blood hemoglobin of suckling pigs of (A, B) outdoor as compared with indoor confinement; C, D, administration of iron and copper salts to the dam during gestation and lactation, and administration of iron and copper salts to the pigs themselves

TABLE 5.—Blood hemoglobin and erythrocyte count, and weights, of a litter of pigs kept indoors

[Litter 93 DJ*; sow and pigs kept inside; no medication]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date	Age of pigs	Pig No.										Average
		93-90-B	93-9-B	93-90-B	93-30-B	93-30-B	93-60-B	93-90-S	93-9-S	93-3-S	93-30-S	
1929												
Apr. 13	2 hours.....	10.19	8.50	12.56	11.83	12.15	11.39	13.92	9.89	10.84	10.03	11.13
15	1 day.....	9.45	7.35	10.47	9.14	9.00	9.40	10.92	8.68	7.94	7.73	9.01
17	4 days.....	5.43	5.83	7.01	-----	6.28	6.31	7.94	5.31	6.96	5.97	6.34
24	1 week 4 days.....	4.99	3.57	5.92	-----	4.72	5.63	5.07	3.46	4.11	4.35	4.45
May 1	2 weeks 4 days.....	3.07	2.11	3.13	-----	3.90	4.12	3.43	3.47	3.05	3.16	3.28
8	3 weeks 4 days.....	3.44	2.17	2.21	-----	3.53	3.06	-----	3.04	2.62	2.63	2.85
15	4 weeks 4 days.....	2.35	2.04	2.06	-----	2.82	3.08	2.97	3.07	2.76	2.19	2.50
22	5 weeks and 4 days.....	2.27	2.79	2.19	-----	2.09	3.53	3.97	3.51	2.75	2.55	2.85

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

May 1	2 weeks 4 days.....	-----	2.23	-----	-----	4.98	-----	4.83	2.87	3.40	2.81	3.53
-------	---------------------	-------	------	-------	-------	------	-------	------	------	------	------	------

WEIGHTS OF PIGS (POUNDS)

Apr. 13	At birth.....	2.3	2.4	2.7	2.4	2.6	2.5	2.5	2.2	2.5	2.4	2.45
May 22	5 weeks 4 days.....	7.2	8.0	13.0	-----	12.2	15.5	17.0	14.0	15.5	12.0	12.71

* Blood of sow 93 DJ contained 11.67 and 11.58 gm. hemoglobin per 100 c. c. when tested on Apr. 3 and Apr. 13, respectively.

The first blood samples, taken from 2 to 10 hours after farrowing, indicate that the pig is born with a fairly high level of hemoglobin, averaging 11 to 11.5 gm. per 100 c. c. A rapid decrease occurs during the first week to a level but little more than half that at birth, and continues for four or five weeks if the pigs are left indoors (litter 93 DJ). Removal to an outside pen, with no access to soil or vegetation, brings about a prompt recovery (litter 90 DJ), both in blood picture and in physical condition. At 39 days of age the litter indoors averaged 17.71 pounds in weight, while at 47 days of age the outdoor litter averaged 27.94 pounds. While part of this difference in weight was due to age and part to a difference in size of litter, there can be little doubt that much of it was the result of the favorable influence of outside conditions. These two litters as they appeared at this time (May 29) are shown in Figure 2.

In order to obtain more evidence concerning the effect of copper and iron medication of suckling pigs on the concentration of hemoglobin in the blood and to determine whether this effect can be secured by treatment of the sow with copper and iron salts preceding parturition and during the suckling period, two litter-mate Duroc-Jersey sows, 96 DJ and 99 DJ, were bred to the same boar. Two weeks before farrowing time sow 96 DJ was given daily doses of ferric citrate (4.0 gm.) and copper sulphate (0.75 gm.) equivalent to 750 mgm. of iron and 191 mgm. of copper daily. The former as a solid and the latter dissolved in 50 c. c. of distilled water, were mixed with the feed. This dose was continued until the litter, farrowed on April 16, 1929, was removed from observation. Sow 99 DJ

received no iron or copper supplements at any time. Blood samples were taken from the individual pigs from two to five hours after birth, after which each litter was divided into two groups, containing four or five pigs each. One group in each litter received daily by mouth water solutions of ferric citrate and copper sulphate in amounts

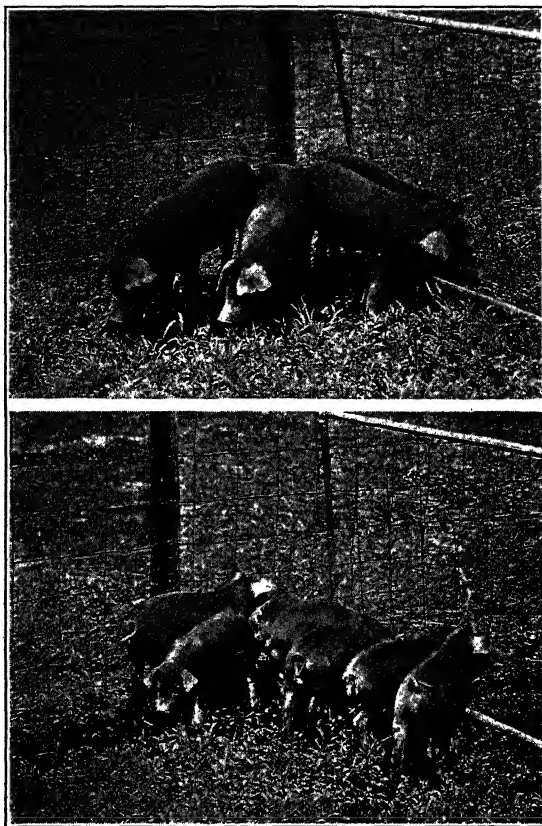


FIGURE 2.—The effect of indoor as compared with outdoor living conditions on the health and vigor of suckling pigs. The four pigs shown at A farrowed in the barn and were removed outdoors to cindered pens in about two weeks; their average weight was about 30 pounds each at the time the photograph was taken. The seven pigs shown at B farrowed in the barn and were kept there until a few days before this photograph was taken, at which time they were almost 2 months old. Long confinement indoors has reduced them to a distinctly anemic condition and at the time the photograph was taken their average weight was only about 14 pounds each. Although litter B was larger than litter A and eight days younger, the contrast in their size and condition must have been caused mainly by the difference between indoor and outdoor conditions of living

containing, respectively, 25 mgm. of iron and 5 mgm. of copper. During the observation of these two litters the little pigs were kept from the dam's feed and feces, either by putting them with the sow only at suckling time (every two or four hours) or, later, by cleaning the pen containing both sow and pigs every two or three hours and feeding the sow in another pen.

TABLE 6.—*Blood hemoglobin, erythrocyte count, and body weight of a litter of pigs half of which was given iron citrate and copper sulphate in individual doses; the sow had also been fed these salts*

[Litter 96 DJ*]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date	Age of pigs	Pigs fed ferric citrate and copper sulphate				Control pigs				Average treated	Average controls
		99-00-B	99-0-S	99-00-B	99-0-B	99-30-B	99-30-S	99-30-S	99-00-S		
1929											
April 16	5 hours.....	9.38	11.16	9.82	10.42	10.79	12.61	10.19	9.34	10.19	10.73
17	30 hours.....	7.99	10.22	8.73	8.74	9.16	8.41	9.53	7.99	8.92	8.77
24	1 week, 1 day.....	7.42	7.91	7.68	6.88	6.29	6.28	7.46	6.90	7.47	6.73
May 1	2 weeks 1 day.....	7.24	7.90	8.06	7.58	4.37	4.71	3.99	4.00	7.69	4.27
8	3 weeks 1 day.....	8.41	8.82	9.00	6.93	4.24	4.01	3.12	3.78	8.29	3.79
15	4 weeks 1 day.....	9.56	10.33	10.11	7.63	3.97	3.77	4.28	5.05	9.41	4.27

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

April 16	5 hours.....	6.67	8.07	6.73	5.71	7.09	8.71	7.09	5.76	6.80	7.16
May 8	3 weeks 1 day.....	6.84	7.43	8.73	5.29	3.76	3.59	4.88	4.13	7.07	4.09

WEIGHTS OF PIGS (POUNDS)

Apr. 16	At birth.....	2.5	2.5	2.6	2.6	2.5	2.4	2.8	2.5	2.55	2.55
May 8	3 weeks 1 day.....	9.5	8.5	10.0	11.0	7.5	8.0	11.0	9.0	9.75	8.87
15	4 weeks 1 day.....	9.0	8.5	10.0	10.5	8.5	10.0	14.0	9.0	9.50	10.37

* Blood of sow 96 DJ contained 12.06, 11.43, and 9.59 gm. hemoglobin per 100 c. c. when tested on Apr. 3, Apr. 15, and Apr. 17, respectively.

^b Died May 16 of unknown cause.

TABLE 7.—*Blood hemoglobin, erythrocyte count, and body weight of a litter of pigs a part of which was given iron citrate and copper sulphate in individual doses, but when the sow had not been fed these salts*

[Litter 99 DJ*]

HEMOGLOBIN (GRAMS PER C. C. OF BLOOD)

Date	Age of pigs	Pigs fed ferric citrate and copper sulphate				Control pigs					Average treated	Average controls
		99-0-B	99-3-B	99-00-S	99-0-S	99-30-S	99-30-B	99-30-S	99-3-S	99-33-B		
1929												
Apr. 17	2 hours.....	12.39	11.14	10.61	8.89	9.86	11.10	12.48	12.73	9.24	10.76	11.08
18	40 hours.....	8.18	7.54	8.09	7.25	8.21	9.17	9.31	9.31	7.27	7.76	8.65
24	1 week.....	8.26	6.26	6.89	8.14	5.54	6.00	6.32	7.24	5.07	7.39	6.03
May 1	2 weeks.....	8.47	6.86	6.38	8.91	4.46	3.71	3.94	4.97	4.09	7.65	4.23
8	3 weeks.....	9.44	9.32	7.52	6.89	3.38	3.35	3.58	4.05	2.88	8.29	3.45
15	4 weeks.....	10.11	8.81	7.99	8.23	2.90	3.24	3.46	4.13	2.44	8.78	3.23
22	5 weeks.....	10.32	10.32	10.81	9.22	3.77	2.91	3.38	3.77	2.77	10.17	3.32

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

Apr. 17	2 hours.....	7.33	6.83	7.53	6.92	6.21	7.65	8.44	7.85	5.93	7.15	7.22
May 15	4 weeks.....	5.95	-----	7.62	7.96	3.18	3.44	4.06	4.80	3.67	7.18	3.83

WEIGHTS OF PIGS (POUNDS)

Apr. 17	At birth.....	2.0	2.3	2.4	2.6	2.0	2.6	2.1	2.5	2.5	2.32	2.34
May 8	3 weeks.....	8.0	10.0	9.0	10.0	8.0	12.0	9.0	10.5	10.0	9.25	9.90
15	4 weeks.....	9.0	11.5	11.0	12.0	10.0	13.0	9.5	14.0	11.5	10.87	11.60
22	5 weeks.....	10.0	12.7	10.2	12.7	10.0	12.7	10.0	15.5	13.0	11.40	12.25

* Blood of sow 99 DJ contained 11.33 and 12.19 gm. hemoglobin per 100 c. c. when tested on Apr. 3 and Apr. 15, respectively.

All of the observations taken on these two litters are recorded in Tables 6 and 7. The hemoglobin results are shown graphically in Figure 1, C and D. The conclusions that may be drawn from these results seem clear cut. In both litters all of the pigs receiving copper and iron medication escaped anemic levels of hemoglobin. Although this treatment did not prevent a marked initial fall in hemoglobin concentration, in no case was a level of 6 gm. per 100 c. c. attained. For all but one pig (9-B in litter 96 DJ) a rapid recovery in hemoglobin occurred, so that at 4 or 5 weeks of age the birth level was again reached. The untreated pigs present an entirely different picture. The drop in hemoglobin concentration continued in every case until a level of 3 or 4 gm. or less per 100 c. c. was reached. It is noteworthy that the untreated pigs nursed by the sow receiving copper and iron medication were not appreciably benefited as judged by comparison with the pigs in the other litter. The effectiveness of the copper and iron treatment of suckling pigs in the prevention of nutritional anemia is clearly evident, as is the ineffectiveness of oral administration of these metals to the lactating sows.

It is interesting to note from Tables 6 and 7 that the body weights of the pigs were not favorably influenced by the copper and iron medication. Evidently the rigid restriction of the pigs to the milk of the dam prevented normal gains in weight.

The individual dosing of anemic pigs under practical farm conditions would of course be out of the question. It is unfortunate that supplementing of the sow's ration with salts of iron and copper—a procedure that would be thoroughly practical—is ineffective in preventing anemia in pigs. In all probability this is due to the fact that the iron and copper content of milk is not readily, if at all, raised by administering iron and copper salts to the lactating female (5, 6).⁶ Another possible method of dosing the pigs was to spread a solution of the metallic salts over the udder of the sow several times a day, so that the little pigs would of necessity remove some copper and iron salts from the teats while suckling.

This expedient was tried in the case of a litter of nine Hampshire pigs, No. 23 Hamp. Two solutions were made up, the first containing 7.86 gm. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per liter and the second 42.8 gm. of ferric citrate per liter. These solutions were applied by hand to the udder of the sow four, five, or more times daily. Starting on June 27 a mixed solution of the salts containing corn sirup in addition was substituted. This solution was made by dissolving 15.72 gm. of hydrated copper sulphate in 500 c. c. of water; the solution was brought to the boiling point, and 85.6 gm. of powdered ferric citrate was added slowly with shaking. To the mixture was then added about 1 pint of corn sirup. This was applied to the udder of the sow by hand. The sow and her litter were kept indoors throughout the experimental period. The observations on this litter comprise Table 8. The hemoglobin results are given graphically in Figure 3, A.

⁶ In unpublished experiments in cooperation with W. B. Nevins, of the Department of Dairy Husbandry, it has been found that daily doses of 3.2 gm. of hydrated copper sulphate given to dairy cows do not appreciably alter the copper content of milk. For a brief description of these experiments, which will be continued and extended to iron salts during the coming year, see 15, p. 120-121.

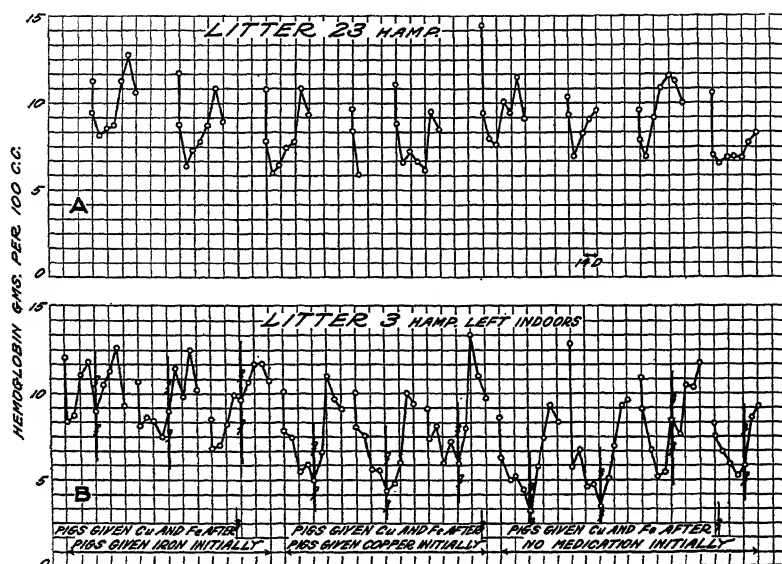


FIGURE 3.—The effect on the blood hemoglobin concentration of suckling pigs of (A) smearing a solution of iron and copper salts on the udder of the dam, and (B) of the separate and combined medication of the pigs with copper and iron salts

TABLE 8.—Blood hemoglobin, erythrocyte count, and weights of a litter of pigs when the sow's udder was covered with a solution of iron citrate and copper sulphate

[Litter 23 Hampshire; kept indoors]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date	Age	Pig No.								
		23-30-B	23-0-B	23-0-B	23-0-B	23-30-S	23-0-S	23-0-S	23-0-S	Average
1929										
June 4	9 hours	11.26	11.74	10.73	9.66	11.06	14.40	10.33	9.62	10.51
5	1 day	9.45	8.73	7.73	8.36	8.59	9.38	9.27	7.82	8.47
12	1 week 1 day	8.10	6.22	5.87	5.87	6.50	7.92	6.89	6.86	6.75
19	2 weeks 1 day	8.53	7.35	6.43	(*)	7.17	7.60	8.18	9.12	6.82
26	3 weeks 1 day	8.74	7.78	7.38		6.55	10.10	9.02	10.78	6.87
July 3	4 weeks 1 day	11.32	8.65	7.81		6.03	9.32	9.50	11.57	6.81
10	5 weeks 1 day	12.62	10.99	10.76		9.44	11.48		11.23	7.66
15	6 weeks 1 day	10.68	8.89	9.22		8.50	9.10		9.93	8.27

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

June 4	9 hours	6.84	7.34	7.08	6.23	6.25	7.55	5.51	5.44	4.69
12	1 week 1 day									3.95

WEIGHTS OF PIGS (POUNDS)

June 4	At birth	3.5	3.4	3.1	3.1	3.4	4.0	3.5	3.0	3.1
5	1 day	4.0	4.1	3.9	3.2	4.0	4.5	4.0	3.5	3.9
12	1 week 1 day	6.5	6.5	6.5	6.0	7.0	7.5	7.0	6.0	6.5
19	2 weeks 1 day	9.5	9.5	10.0	(*)	11.0	11.0	11.0	8.5	11.5
26	3 weeks 1 day	10.5	12.0	14.0		13.5	13.5	14.0	10.0	13.0
July 3	4 weeks 1 day	12.0	14.0	17.0		16.0	18.0	13.5	16.0	
10	5 weeks 1 day	14.0	19.0	18.5		19.5	20.5		15.0	18.5
15	6 weeks 1 day	16.5	24.0	24.5		24.0	26.0		21.0	23.5

* Dead.

* Killed by sow.

The pigs in this litter increased in body weight at a rate that was not far from normal. The hemoglobin concentration of the blood decreased, as usual, for the first week or so, after which it increased

up to the fifth week of age, sometimes to a value higher than that initially observed. A comparison of the hemoglobin curves of this litter with those of litter 93 DJ (fig. 1, B) seems to indicate clearly that the method of administering copper and iron salts to suckling pigs by spreading a solution of the salts over the udder of the dam is an effective as well as a practical method of preventing milk anemia when, because of inclement weather, the pigs must be kept indoors. The experiment unfortunately did not continue long enough to evaluate the significance of the decrease in hemoglobin concentration, generally slight, that occurred in six of the eight surviving pigs from the fifth to the sixth weeks of age.

In the last experiment a litter of 10 Hampshire pigs was divided into three groups in order to determine the effect on the concentration of hemoglobin of the separate administration of ferric citrate and copper sulphate. Three of the pigs received ferric citrate in the ordinary dosage (equivalent to 25 mgm. of iron daily) three received copper sulphate (equivalent to 5 mgm. of copper daily) and four were untreated. At 4 weeks of age all pigs received the combined treatment of iron and copper. The experiment is not particularly conclusive, except in showing again the markedly favorable effect of the combined dosage. The hemoglobin changes are presented in Figure 3, B. All of the pigs receiving iron showed the initial drop in hemoglobin, but all of them showed some response to the iron medication. In two cases the response was marked. The sample of ferric citrate used contained 0.73 mgm.⁷ of copper per gram, so that in this group each pig received 0.01 mgm. of copper daily in its dose of ferric citrate. The three pigs receiving copper sulphate only were not benefited appreciably by this treatment. All pigs responded promptly to the combined dosage of iron and copper.

SUMMARY

A few hours after birth the hemoglobin concentration in the blood of pigs was found to range from less than 9 to almost 15 gm. per 100 c. c., averaging 10.75 gm. in the 54 pigs tested. There is a rapid decrease in hemoglobin during the first few days, a decrease which starts at or soon after birth.

In the case of litters remaining indoors, this decrease in the level of hemoglobin in the blood continued until concentrations of 4, 3, or even 2 gm. per 100 c. c. of blood were reached. In the case of litters removed outdoors to cinderbed pens, this decrease was stopped and an increase was induced until the birth level of hemoglobin was again reached.

The birth level of hemoglobin could be restored also by administering ferric citrate and copper sulphate to the pigs, either by pipette in daily doses equivalent to 25 mgm. of iron and 5 mgm. of copper, or by spreading a solution of these metallic salts over the udder of the lactating sow. However, the administration of copper and iron salts to the dam during the last two weeks of gestation and the period of lactation had no appreciable effect on the blood hemoglobin concentration of the suckling pigs.

The administration of copper sulphate alone to the suckling pigs was ineffective in promoting the regeneration of hemoglobin. Ferric citrate alone, contaminated with a very small amount of copper, was

⁷ Determined by the potassium ethyl xanthate method.

appreciably effective, but much less so than a combination of copper sulphate and ferric citrate.

LITERATURE CITED

- (1) CARROLL, W. E., and MITCHELL, H. H.
1927. RESULTS OF FEEDING COPPERAS IN PAIRED FEEDING EXPERIMENTS WITH GROWING SWINE. *Amer. Soc. Anim. Prod. Proc.* 1927: 73-82.
- (2) ——— MITCHELL, H. H., and HUNT, G. E.
1930. FERRIC CITRATE AS AN INGREDIENT OF MINERAL MIXTURES IN PAIRED-FEEDING EXPERIMENTS WITH GROWING SWINE. *Jour. Agr. Research* 40: ———.
- (3) DORLENCOURT and GALUGAREANU-NANDRIS.
1926. [DETERMINATION AND VARIATIONS OF THE IRON CONTENT OF BREAST MILK.] *Bul. Soc. Pediatr. Paris* 24: 376. *Abst. in Chem. Abs.* 22: 983. 1928.]
- (4) DOYLE, L. P., MATHEWS, F. P., and WHITING, R. A.
1927. ANEMIA IN YOUNG PIGS. *Ind. Agr. Expt. Sta. Bul.* 313, 18 p., illus. [Also in *Jour. Amer. Vet. Med. Assoc. (n. s.)* 25: 491-510. 1928.]
- (5) ELVEHJEM, C. A., HERRIN, R. C., and HART, E. B.
1927. IRON IN NUTRITION. III. THE EFFECTS OF DIET ON THE IRON CONTENT OF MILK. *Jour. Biol. Chem.* 71: 255-262.
- (6) ——— STEENBOCK, H., and HART, E. B.
1929. THE EFFECT OF DIET ON THE COPPER CONTENT OF MILK. *Jour. Biol. Chem.* 83: 27-34.
- (7) HART, E. B., ELVEHJEM, C. A., STEENBOCK, H., BOHSTEDT, G., and FARGO J. M.
1929. ANEMIA IN SUCKLING PIGS. *Wis. Agr. Expt. Sta. Bul.* 409, 14 p., illus.
- (8) ——— STEENBOCK, H., ELVEHJEM, C. A., and WADDELL, J.
1925. IRON IN NUTRITION. I. NUTRITIONAL ANEMIA ON WHOLE MILK DIETS AND THE UTILIZATION OF INORGANIC IRON IN HEMOGLOBIN BUILDING. *Jour. Biol. Chem.* 65: 67-80.
- (9) ——— STEENBOCK, H., WADDELL, J., and ELVEHJEM, C. A.
1928. IRON IN NUTRITION. VII. COPPER AS A SUPPLEMENT TO IRON FOR HEMOGLOBIN BUILDING IN THE RAT. *Jour. Biol. Chem.* 77: 797-812, illus.
- (10) McGOWAN, J. P.
1924. ON THE PATHOLOGY OF IRON DEFICIENCY AND COTTONSEED POISONING IN PIGS. *Jour. Path. and Bact.* 27: 201-209, illus.
- (11) ——— and CRICHTON, A.
1924. IRON DEFICIENCY IN PIGS. *Biochem. Jour.* 18: [265]-282.
- (12) MCHARGUE, J. S., HEALY, D. J., and HILL, E. S.
1928. THE RELATION OF COPPER TO THE HEMOGLOBIN CONTENT OF RAT BLOOD. PRELIMINARY REPORT. *Jour. Biol. Chem.* 78: 637-641.
- (13) MATHEWS, F. P., DOYLE, L. P., and WHITING, R. A.
1929. THE EFFECT OF ULTRA-VIOLET RADIATION ON BLOOD FORMATION IN YOUNG PIGS. *Amer. Jour. Physiol.* 88: 616-619.
- (14) MITCHELL, H. H.
1929. MINERAL DEFICIENCIES IN SWINE RATIONS. *Jour. Amer. Vet. Med. Assoc. (n. s.)* 27: 74: 651-661.
- (15) MUMFORD, H. W.
1929. A YEAR'S PROGRESS IN SOLVING FARM PROBLEMS OF ILLINOIS. LIVESTOCK INVESTIGATIONS 1928-29. III. *Agr. Expt. Sta. Ann. Rpt.* 42: 120-121.
- (16) NEWCOMER, H. S.
1919. ABSORPTION SPECTRA OF ACID HEMATIN, OXYHEMOGLOBIN, AND CARBON MONOXIDE HEMOGLOBIN. A NEW HEMOGLOBINOMETER. *Jour. Biol. Chem.* 37: 465-496, illus.
- (17) SKINNER, J. T., and PETERSON, W. H.
1928. THE IRON AND MANGANESE CONTENT OF FEEDING STUFFS. *Jour. Biol. Chem.* 79: 679-687.
- (18) VAN SLYKE, D. D., and STADIE, W. C.
1921. THE DETERMINATION OF THE GASES OF THE BLOOD. *Jour. Biol. Chem.* 49: 1-42, illus.
- (19) WADDELL, J., STEENBOCK, H., ELVEHJEM, C. A., and HART, E. B.
1928. IRON IN NUTRITION. V. THE AVAILABILITY OF THE RAT FOR STUDIES IN ANEMIA. *Jour. Biol. Chem.* 77: 769-775.

LINKAGE STUDIES WITH "SLASHED" AND "GLOSSY₁" OF THE "BN" LINKAGE GROUP IN MAIZE¹

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INTRODUCTION

Maize furnishes unusually satisfactory material for the study of linkage relations in plants. Recessive characters which can be differentiated in the seedling state are especially valuable in linkage studies. The character "slashed," reported in this paper, was first observed in seedling progenies of three selfed ears of a 3-year selfed strain of Minnesota No. 13 yellow dent corn. These progenies were grown in the greenhouse during the winter of 1922-23. Apparently the same character has appeared in a number of unrelated selfed strains of Minnesota No. 13 since that time. Lindstrom (?)² reported a character which he described as "slashed or shredded seedling," and R. A. Emerson provided the writer with material described as "slashed." In the studies conducted at University Farm, St. Paul, there did not appear to be any resemblance between the type obtained from Emerson and the slashed seedling character reported in this paper.

It was early learned that the character slashed was linked with a glossy character obtained from Rustler white dent, which was found to be "glossy₁" of the "Bn" group. Crosses were made for the purpose of supplementing the available data (6) concerning the independence of the Bn group from other linkage groups. Data on the inheritance of the slashed character and linkage studies of slashed and "glossy₁" in relation to characters in the established linkage groups are presented in this paper.

DESCRIPTION OF SLASHED

Slashed is a leaf character. Its appearance is characterized by long, thin, chlorophyll-deficient striae on each leaf of the plant, usually in greater abundance near the tip. (Fig. 1.) These thin spots break through in later stages of plant development and give the leaf a slashed appearance. Slashed plants are usually much less vigorous than normal plants (fig. 2) and generally somewhat later in maturing. In a favorable season small ears may be obtained from most slashed plants. The character is generally very definite and easy to distinguish at any stage from the seedling to the mature plant. In a very few of the crosses studied, however, slashed plants were almost as vigorous as normal ones, and classification was more difficult. This was particularly true of segregates from the tunicate versus slashed cross.

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² Reference is made by number (italic) to "Literature cited," p. 950.

INHERITANCE OF SLASHED

Data for the segregation of normal and slashed plants in several selfed lines of Minnesota No. 13 are summarized in Table 1.

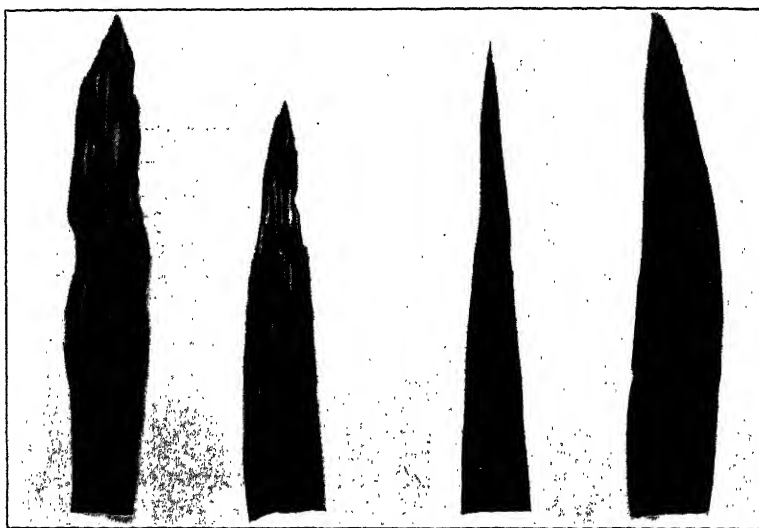


FIGURE 1.—The first two seedling leaves of slashed (left) and normal (right) maize plants

TABLE 1.—Segregation for slashed plants in Minnesota No. 13 selfed strains

Strain No.	Years selfed	Number of plants	
		Normal	Slashed
42-2.....	3	17	3
42-4.....	3	64	20
42-5.....	3	61	27
9-1.....	1	61	20
1-8.....	1	15	4
1-32.....	1	121	39
30-1.....	3	303	102
30-2.....	3	15	4
Total.....		657	219
Expected for 3 : 1 segregation.....		657	219

There were 657 normal plants and 219 slashed, which agrees perfectly with a 3:1 ratio. This indicates that slashed is a simple recessive character. In the F_2 generations of certain crosses made for the purpose of determining the linkage relations of slashed, however, the obtained segregation approached a 13:3 ratio, which suggested the possibility of two factor pairs. One might assume the complementary action of a recessive factor with a dominant factor necessary for the production of slashed plants. With both factor pairs segregating, 2 of every 3 slashed F_2 plants when selfed would be expected to segregate in the ratio of 3 slashed to 1 normal. A large number of self-pollinations were made on slashed plants in culture A4a, an F_2 generation which segregated in approximately a 13:3 ratio. (Tables 2 and 4.) Selfed ears were obtained on 24 plants, although the number of seed obtained was rather small in some cases. The data for the F_2 segregation and for the F_3 progenies of selfed F_2 ears are presented in Table 2.



FIGURE 2.—Slashed maize plant (left) and normal plant (right) from a segregating line just before silking

TABLE 2.—Breeding behavior of normal and slashed plants in the F_2 generation, and in the F_3 progenies of selfed slashed plants

Description		Normal	Slashed
F_2 culture A4a.....	{ Obtained.....	1,863	421
	{ Expected 13: 3.....	1,856	428
	{ 13 lines.....		(^a)
	{ 2 lines.....	(^b)	(^b)
F_3 progenies.....	{ 1 line.....	1	41
	{ do.....	3	32
	{ do.....	2	5
	{ do.....	2	3

^a All.

^b Too weak to classify.

Only 4 of the 24 selfed ears obtained from F_2 slashed plants produced progeny which contained any normal seedlings, while 2 of these gave too few normals to be considered as probable 3:1 ratios. From

the F_3 line which segregated for 2 normal and 3 slashed plants, 1 normal plant was selfed. On greenhouse test, the progeny of this plant consisted of 75 normal and 20 slashed plants, a deviation of 4 ± 2.5 from a 3:1 ratio. It is evident that this normal plant carried a recessive factor for slashed, which indicates that it resulted from accidental cross-pollination. In case two factors were necessary for the expression of slashed in culture A4a, one would expect the progeny of 16 slashed plants out of the 24 to segregate in a ratio of 3 slashed to 1 normal. It appears obvious that the few normal seedlings obtained were the result of off-pollination, and that the deviation from a 3:1 ratio obtained in culture A4a can not be explained by a factorial hypothesis which assumes the interaction of a dominant and a recessive factor necessary for the expression of slashed. In culture A4a (see summary of F_2 data, Table 4) and in several other crosses reported in this paper, the percentage of slashed seedlings is less than expected on the basis of a 3:1 ratio of normal to slashed, this being, apparently, for the most part, due to the deficiency of slashed in the double recessive class. From a careful study of all of the data, it is concluded that slashed is a simple recessive character, and the factor pair for normal versus slashed is designated *Ssl*.

STUDIES OF SLASHED IN RELATION TO FACTORS IN KNOWN LINKAGE GROUPS

Slashed has been studied in relation to one or more factors in each of the eight known linkage groups. The linkage value in most cases was calculated in terms of p , or the crossover value expressed as a decimal fraction, which was obtained by the use of Yule's coefficient of association. (See Collins (1).) Probable errors for p were computed from tables prepared by F. R. Immer of this division.

An alphabetic list of the factors which were studied in relation to *sl* is given below. References to publications for the characters listed may be found in Hayes and Garber (4). The factor symbols for dominant characters are capitalized.

- A. Anthocyan, general plant color found in aleurone, pericarp, stems, leaves, etc.
- br. Brachytic, characterized by shortened internodes.
- Bn. Brown aleurone.
- C. Colored aleurone, complementary to A and R.
- Fl. Flinty endosperm, fl floury.
- g. Golden plant color.
- gl. Glossy leaf.
- lg. Liguleless leaf.
- P. Pericarp and cob color. An allelomorphic series P^{rr} , P^{wr} , etc.
- Pr. Purple aleurone.
- R. Colored aleurone, complementary to A and C.
- ra. Ramose ear.
- sh. Shrunken endosperm.
- su. Sugary endosperm.
- ts_1 , ts_2 . Tassel seed. Produces only pistillate flowers in the tassel.
- Tu. Tunicate ear.
- wx. Waxy endosperm.
- Y. Yellow endosperm.

The value of p for obtained F_2 ratios involving the complementary factors A, C, and R in relation to *sl* was calculated from formulae presented by Owen (8). By this method, the degree of association in inheritance is expressed by means of a product moment coefficient of

correlation r ; and the p or crossover value is calculated from r for various phenotypic ratios. The summarized F_2 data for these studies are presented in Table 3.

TABLE 3.—Summary of F_2 data for crosses of sl with the A , C , and R aleurone factors

Culture No.	Colored		Colorless		Total	Per cent slashed		r	Factor relations	p
	Sl	sl	Sl	sl		Colored	Colorless			
H 46.....	603	161	824	249	1,837	21.1	23.2	0.025 ± 0.016	{Coupling C or R Repulsion A Coupling C or R	0.463
H 175.....	1,022	258	896	287	2,463	20.2	24.3	$.049 \pm .014$.537 .446
$r/P. E.$						H 46=1.6; H 175=3.5.				

In each cross, the colored seeds produced a smaller percentage of slashed plants than did the colorless. In view of the fact that the coupling phase for the C and R factors in relation to sl was studied in each case, the data suggest the possibility of a weak linkage. The apparent association, however, is very slight, as indicated by a correlation coefficient of 0.025 ± 0.016 for H 46 and 0.049 ± 0.014 for H 175. In no case were more than 25 per cent slashed plants obtained from the colorless seeds, and when considered in relation to other linkage data in the A , C , and R , linkage groups, it seems probable that sl is inherited independently of A , C , and R .

LINKAGE STUDIES WITH THE C GROUP

The repulsion phase involving F_2 segregation for sl in relation to sh and wx was studied. The summarized data are presented in Table 4.

TABLE 4.—Summary of F_2 data for crosses of sl with sh and wx of the C group

Culture No.	Sh		sh		Total	Per cent slashed		p
	Sl	sl	Sl	sl		Sh	sh	
H 175.....	1,457	424	461	121	2,463	22.5	20.8	
H 173.....	265	82	61	18	426	23.6	22.8	
Total.....	1,722	506	522	139	2,889			0.487 ± 0.010
	Wx		wx			Wx	wx	
A 4a.....	1,434	364	429	57	2,284	20.2	11.7	$.409 \pm .012$
H 176A.....	1,157	366	349	102	1,974	24.0	22.6	$.489 \pm .012$
Total.....	2,591	730	778	159	4,258			$.455 \pm .008$

With the exception of culture A 4a, the data indicate independent inheritance of sl with the sh and wx factors. In culture A 4a, there is a deficiency of slashed for a 3 : 1 ratio of normal to slashed, the deficiency being chiefly in the double recessive class. The deviation of p from 0.50 for independent inheritance is 2.3 times the probable error for the $sl-sh$ crosses and 5.6 times the probable error for the total data for the $sl-wx$ crosses. This rather large deviation from the

expected in the latter case appears to be principally due to the deficiency in the double recessive class in cross A 4a, the p value of 0.489 ± 0.012 for H 176A clearly indicating independent inheritance for the factors wx with sl . When the data for these crosses are considered in relation to those presented for the A , C , and R factors, it appears very probable that sl is independent in inheritance of C , sh , and wx factors. The relation of wx and sl will, however, be studied further in back-cross material.

LINKAGE STUDIES WITH THE R GROUP

The relation of sl with g was studied in the repulsion phase in one cross. A summary of the data obtained is presented in Table 5.

TABLE 5.—Summary of F_2 data for a cross of sl with g of the R group

Culture No.	G		g		Total	Per cent slashed		p
	Sl	sl	Sl	sl		G	g	
H 176B.....	948	301	290	45	1,584	24.1	13.4	0.400 ± 0.014

There appears to have been considerable elimination of double recessives since only 13.4 per cent of the golden plants were slashed, whereas the normal green plants segregated in approximately a 3:1 ratio of nonslashed to slashed. When considered in relation to the data presented for the A , C , and R factors it is apparent that sl is probably independent of the R and g factors in this group. The g - sl relation will be further tested, however, in back-cross material.

LINKAGE STUDIES WITH THE SU GROUP

F_2 generations of four crosses involving segregation for su and sl in the repulsion phase were studied. The origin of the su factor was different in three crosses. Intercrosses of these three sugary parents were made, and only sugary kernels were obtained in each case, indicating only one su factor concerned in all crosses. The relation of Tu and sl was studied in the progeny of tunicate plants obtained from a cross of $Tutu Slsl \times tutu slsl$. A summary of the data for these crosses is presented in Table 6.

TABLE 6.—Summary of F_2 data for crosses of sl with su and Tu of the su group

Culture No.	Su		su		Total	Per cent slashed		p
	Sl	sl	Sl	sl		Su	su	
A 9a and b.....	1,498	473	321	33	2,325	24.0	9.3	0.345 ± 0.012
A 11a and b.....	1,533	528	362	91	2,514	25.6	20.1	$.450 \pm .011$
H 46.....	875	281	273	57	1,486	24.3	17.3	$.440 \pm .014$
H 180.....	492	182	168	25	867	27.0	13.0	$.373 \pm .019$
Total.....	4,398	1,464	1,464	206	7,192	$.416 \pm .006$
A 9a&b.....	Tu		tu		600	Per cent slashed		p
	Sl	sl	Sl	sl		Tu	tu	
	363	69	143	25		16.0	14.9	0.512 ± 0.021

The p value for the sugary slashed crosses varies from 0.345 to 0.456 in direct relation to the deficiency of the double recessive class. The percentage germination for the four crosses, respectively, are Su , 95.0, 93.3, 99.1, and 100.0; and su , 49.3, 60.8, 86.4, and 61.3. The percentage of slashed plants obtained from starchy seeds in each cross approached 25.0, indicating a 3:1 ratio and independence of the factors su and sl . Too few slashed plants were obtained from both nontunicate and tunicate groups of culture A9a and A9b. This deficiency was similar in both groups and there appears to be independent inheritance of the factors Tu and sl . It is a fact of considerable interest that the slashed plants obtained from the tunicate-slashed cross were almost as vigorous as nonslashed plants.

LINKAGE STUDIES WITH THE B GROUP

A study was made of sl in relation to ts_1 and lg in F_2 material and of sl with lg in back-cross material (culture H188). The factors entered in the repulsion phase in each case. A summary of the data is presented in Table 7.

TABLE 7.—Summary of F_2 data for sl with ts_1 and lg , and back-cross data with lg [The factors ts_1 and lg are members of the B group]

Culture No.	Ts_1		ts_1		Total	Per cent slashed		p
	Sl	sl	Sl	sl		Ts_1	ts_1	
H 178.....	677	156	156	52	1,041	18.7	25.0	0.552±0.015
	Lg		lg			Lg	lg	
A 2a.....	381	111	129	41	662	22.6	24.1	.512±.019
H 188.....	97	92	112	105	406	48.7	48.4	.498±.017

The factors lg and ts_1 are located at opposite ends of the chromosome. When considered in relation to this fact, the results obtained indicate slashed to be independent of factors in the B group.

Possible linkage relations between flinty-floury endosperm and slashed were studied. In F_2 flinty-floury segregates in a 1:1 ratio. A linkage of the factor which differentiates flinty from floury with a glossy seedling factor gl_2 was reported by Hayes and Brewbaker (4). Flinty-floury and gl_2 were recently found linked with lg . The summarized data for an F_2 generation segregating for flinty versus floury and normal versus slashed are given in Table 8.

TABLE 8.—Summary of F_2 data for a cross of slashed flinty with nonslashed floury

Data for culture No. H173	Flinty		Floury		Total	Per cent slashed	
	Sl	sl	Sl	sl		Flinty	Floury
Observed.....	983	274	1,007	270	2,534	21.8	21.1
Calculated.....	987	270	1,003	274			
Difference.....	4	4	4	4			

$\chi^2=0.15$; P =very large.

As a test for linkage χ^2 was used. The theoretical population was obtained from the observed ratio rather than from the calculated on the basis of a 1 : 1 segregation for flinty to floury and a 3 : 1 segregation for normal and slashed (Collins, (1)). The value of P , which was used as a means of testing the agreement of observed with a calculated ratio, assuming independent segregation, is very large, indicating a very good fit. In a previous paper (4), it was stated that flinty-floury was independent in inheritance of Gl_1gl_1 and $Rara$. As sl is linked with factors in the Bn linkage group, it should be inherited independently of the factor pair for flinty-floury.

LINKAGE STUDIES WITH THE Y GROUP

Slashed was studied in relation to the Y factor in F_2 generations of six crosses, the Y factor in each case coming from the slashed parent. The summarized data are presented in Table 9.

TABLE 9.—Summary of F_2 data for crosses of sl with Y of the Y group

Culture No.	Y		y		Total	Per cent slashed		p
	Sl	sl	Sl	sl		Y	y	
A 11a.....	591	193	207	59	1,050	24.6	22.2	
H 44.....	829	259	316	102	1,506	23.8	24.4	
A 9a.....	679	229	255	69	1,232	25.2	21.3	
H 176A.....	781	239	249	90	1,369	23.4	26.5	
H 176B.....	920	258	329	77	1,584	21.9	19.0	
H 173.....	1,268	333	434	143	2,178	20.8	24.8	
Total.....	5,068	1,511	1,790	540	8,909	23.0	23.2	0.504±0.005

The results obtained are similar for each of the crosses studied and indicate independent inheritance of the sl and Y factors.

LINKAGE STUDIES WITH THE P GROUP

Studies were made with F_2 generation material involving sl in the repulsion phase in relation to br and ts_2 , and the coupling phase with P for cob color. The summarized data are presented in Table 10.

TABLE 10.—Summary of F_2 data for crosses of sl with br , ts_2 , and P of the P group

Culture No.	Br		br		Total	Per cent slashed		p
	Sl	sl	Sl	sl		Br	br	
H 175.....	690	217	180	62	1,149	23.9	25.6	0.512±0.015
	Ts_2		ts_2			Ts_2	ts_2	
H 172.....	504	157	159	44	864	23.8	21.7	.483±.018
	P		p			P	p	
A 11a and b.....	752	130	275	42	1,199	14.7	13.2	.518±.015

These data indicate independent inheritance of the characters involved.

LINKAGE STUDIES WITH THE A GROUP

Segregation for the *A* factor, as indicated by seedling stem color, was studied in the repulsion phase in two crosses in relation to slashed. The results are summarized in Table 11.

TABLE 11.—Summary of F_2 data for a cross of *sl* with *A* of the *A* group

Culture No.	<i>A</i>		<i>a</i>		Total	Per cent slashed		<i>p</i>
	<i>Sl</i>	<i>sl</i>	<i>Sl</i>	<i>sl</i>		<i>A</i>	<i>a</i>	
H 44.....	820	246	268	100	1,434	23.1	27.2	0.530±0.013
H 46.....	817	223	610	184	1,834	21.4	23.2	.514±.012

Slashed appears to be independent in inheritance of the *Aa* factor pair.

LINKAGE RELATIONS WITH THE *Bn* GROUP

Definite linkage values have been obtained with slashed and characters whose factor locations are in the *Bn* group. The factors *gl₁*, *ra*, and *Bn* were studied in relation to *sl*. It has been possible to study only F_2 generation material up to the present time. The characters entered in the repulsion phase in each cross. The summarized data are presented in Table 12.

TABLE 12.—Summary of F_2 data for crosses of *sl* with *gl₁*, *ra* and *Bn* of the *Bn* group

Culture No.	<i>Gl₁</i>		<i>gl₁</i>		Total	Per cent slashed		<i>p</i>
	<i>Sl</i>	<i>sl</i>	<i>Sl</i>	<i>sl</i>		<i>Gl₁</i>	<i>gl₁</i>	
A 5a.....	181	50	94	2	327			
H 173.....	1,332	546	709	16	2,603			
Total.....	1,513	596	803	18	2,930	28.3	2.2	0.163±0.012
	<i>Ra</i>		<i>ra</i>			<i>Ra</i>	<i>ra</i>	
H 174.....	1,127	492	510	22	2,151	30.4	4.1	.210±.014
	<i>Bn</i>		<i>bn</i>			<i>Bn</i>	<i>bn</i>	
H 44.....	214	102	102	0	418			
A 9a.....	185	69	70	1	325			
H 174.....	135	55	61	4	255			
H 180.....	454	202	207	5	868			
Total.....	988	428	440	10	1,866	30.2	2.2	.157±.015

Since there appears to have been considerable zytotic elimination in many of the slashed crosses, particularly in the double recessive groups, crossover values can be considered only as giving rough approximations of the location of the factors in the chromosome. Crossover percentages of 38.2 for *Bn* versus *ra*, and 18.7 and 29.4 for *Bn* versus *gl₁* were reported by Kvakan (6). The crossover percentages of 16.3 ± 1.2 for *sl* versus *gl₁*, 21.0 ± 1.4 for *sl* versus *ra*, and 15.7 ± 1.5 for *sl* versus *Bn* were obtained by using Yule's coefficient of association method. When calculated by Haldane's method (2), the following crossover percentages were obtained: 16.2 for *sl* versus *gl₁*,

21.0 for *sl* versus *ra*, and 16.6 for *sl* versus *Bn*. These data indicate the order of the genes to be as follows: *ra-gl₁-sl-Bn*.

A further study of the relationship of *sl* with factors in this linkage group is being made in back-cross material in order to determine with greater accuracy the location of the *sl* factor.

LINKAGE STUDIES OF SLASHED WITH THE PR FACTOR

Segregation for the *Pr* aleurone factor was studied in the repulsion phase in relation to *sl* in three crosses, the summarized results being presented in Table 13.

TABLE 13.—Summary of F_2 data for crosses involving segregation for *sl* and *Pr*

Culture No.		Purple		Red		Total	Per cent slashed		<i>p</i>
		<i>Sl</i>	<i>sl</i>	<i>Sl</i>	<i>sl</i>		Purple	Red	
H 46	Observed	372	111	231	50	704	23.0	17.8	0.455±0.019
	Calculated	381	102	222	59				
	Difference	9	9	9	9				
H175	Observed	647	176	375	82	1,280	21.4	17.9	.469±.015
	Calculated	657	166	365	92				
	Difference	10	10	10	10				
A 49	Observed	734	120	242	53	1,149	14.1	18.0	.541±.014
	Calculated	725	129	251	44				
	Difference	9	9	9	9				

In cultures H 46 and H 175, too many red kernels were obtained to approximate a 3:1 ratio, the observed ratios of purple to red being 1.71:1 and 1.79:1, respectively. These ratios are similar to those reported by Hayes and Brewbaker (8). The χ^2 goodness of fit method was used to test for independent inheritance. The *P* values for the three crosses are: 0.10, 0.15, and 0.09 respectively. It may be said that a worse fit could be expected on the basis of random sampling in from 9 to 15 out of 100 trials for these crosses. The crossover value was also calculated in terms of *p*. In no case was the deviation from 0.500 as great as three times the probable error. The results indicate that the *Pr* and *sl* factors are probably inherited independently.

LINKAGE STUDIES OF GL₁ WITH OTHER FACTORS

Linkage studies of a glossy character, obtained from a selfed line of Rustler white dent, were started in 1923. It was later learned from crosses with Brunson's *gl₁* that the glossy factor in the Rustler strain was the same as *gl₁*. The linkage studies were completed and are reported here since they further verify the independence of the *Bn* group from other linkage groups. In all the studies made F_2 data were used, the linkage values being calculated by Yule's coefficient of association method (1) except those for cultures H 159 and H 160, where more than one factor segregated for aleurone color. For these crosses, Owen's coefficient of correlation method was used (8). The data for *gl₁* in relation to the aleurone factors *Pr*, *A*, *C*, and *R* are summarized in Table 14.

TABLE 14.—Summary of F_2 data for crosses of gl_1 with the aleurone factors A , C , R , and Pr

Culture No.	Purple		Red		Total	r	$r/P.E.$	p	Factor relations
	Gl	gl	Gl	gl					
H 159.....	250	78	142	40	510			0.486±0.023	Repulsion Pr .
H 160.....	778	245	260	75	1,358			.487±.014	Repulsion Pr .

Culture No.	Colored		Colorless		Total	r	$r/P.E.$	p	Factor relations
	Gl	gl	Gl	gl					
H 159.....	392	118	522	175	1,207	0.023±0.019	1.2	$A=0.533$	Repulsion A .
H 160.....	1,038	320	936	291	2,585	.002±.013	0.1	$C\&R=.407$.498	Coupling $C\&R$. Coupling $C\&R$.

The value of p closely approaches 0.500 in each case, indicating independent inheritance of gl_1 and the aleurone factors Pr , A , C , and R .

The factor gl_1 was studied in relation to the factors wx , sh , g , su , Tu , Y , and P , representing five linkage groups. The data for these crosses are presented in Table 15.

TABLE 15.—Summary of F_2 data for crosses of gl_1 with other factors in known linkage groups

Culture No.	Gl_1		gl_1		Total	p	Factors studied	Phase ^b	Linkage group
	X^a	x^a	X^a	x^a					
14b.....	201	79	80	36	396			R	C
17a.....	178	42	60	17	297			R	
H 160.....	1,235	426	396	129	2,186			R	
H 161.....	405	156	157	52	770			R	
Total.....	2,019	703	693	234	3,649	0.497±0.008	wx		
H 165.....	269	90	109	44	512			R	
H 167.....	727	245	265	68	1,305			R	
Total.....	996	335	374	112	1,817	.483±.012	sh		
13a.....	308	242	258	73	1,381	.493±.014	g	R	R
10b, 10c, 17a.....	943	151	326	69	1,499			R	su
H 164.....	220	55	79	31	385			R	
Total.....	1,163	206	405	100	1,874	.548±.010	su		
17b.....	194	83	59	22	358	.519±.027	Tu	C	
H 173.....	954	385	365	126	1,830	.522±.012	Y	C	Y
13a.....	426	145	108	43	722	.478±.018	P^c	C	P

^a X =dominant; x =recessive.^b R =repulsion; C =coupling.^c P =Cob color.

The data indicate independence of gl_1 and other factors considered. In only one case does deviation from 0.50 exceed the probable error by more than twice its value. In the su versus gl_1 cross the deviation from 0.50 was 4.8 times as great as the probable error. There is a deficiency of numbers in both su classes. In crosses 10b, 10c, and 17a the germination of the sugary seeds was low, the relative percentage germination for starchy and sugary seeds being 81.9 and 42.6, respectively. In the cross H 164, there was a lack of sugary seeds

in the F_2 generation, the segregation being 322 starchy to 88 sugary. This is a deviation of 14.5 ± 5.9 from the expected 3:1 ratio. The germination percentage for starchy and sugary seeds in this cross was not greatly different, being 92.9 and 97.7, respectively.

SUMMARY AND CONCLUSIONS

A seedling and plant character known as "slashed" was obtained as a segregate from several selfed lines of Minnesota No. 13 yellow dent corn. In these lines, slashed appeared to be a simple recessive, a perfect fit of actual and expected having been obtained. In certain crosses studied later, far too few slashed plants were obtained for a single-factor difference. It was shown in one case, where the results obtained in F_2 closely approximated a 13:3 ratio, that a factorial hypothesis which would give such a ratio did not explain the results. In view of this, and the fact that in several crosses the double recessive class was greatly reduced, it is concluded that slashed is a simple recessive, and the factor pair concerned has been designated *Slsl*.

Segregating generations involving the factor for slashed in relation to factors in each of the eight well-known linkage groups, as well as *Prpr*, whose definite linkage relations are not known, have been studied for the most part in F_2 material. In all cases, except for factors in the *Bn* group, the data indicate probable independent inheritance.

The independence of *sl* and other members of the *Bn* group from other linkage groups is further verified by F_2 linkage studies of *gl_1* in relation to factors in the *C*, *R*, *su*, *Y*, *P*, and *A* groups in which independent inheritance is indicated in every case.

Studies of the linkage relations of *sl* with *gl_1*, *ra*, and *Bn* of the *Bn* group were made with F_2 material, the following crossover values being obtained by Yule's coefficient of association method: *sl* versus *gl_1*, 16.3 ± 1.0 ; *sl* versus *ra*, 21.0 ± 1.4 ; and *sl* versus *Bn*, 15.7 ± 1.5 . When considered in relation to the data reported by Kvakan (6), the order of the genes appears to be *ra-gl_1-sl-Bn*.

The results of these studies further support those of an earlier investigation in indicating that the *Bn* linkage group is independent of other groups.

LITERATURE CITED

- (1) COLLINS, G. N.
1924. MEASUREMENT OF LINKAGE VALUES. Jour. Agr. Research 27: 881-891.
- (2) HALDANE, J. B. S.
1919. THE PROBABLE ERRORS OF CALCULATED LINKAGE VALUES, AND THE MOST ACCURATE METHOD OF DETERMINING GAMETIC FROM CERTAIN ZYGOTIC SERIES. Jour. Genetics 8: [291]-297.
- (3) HAYES, H. K., and BREWBAKER, H. E.
1926. FACTORS FOR COLOR OF ALEURONE AND ENDOSPERM IN MAIZE. Jour. Amer. Soc. Agron. 18: 761-767.
- (4) ——— and BREWBAKER, H. E.
1928. GLOSSY SEEDLINGS IN MAIZE. Amer. Nat. 62: 228-235.
- (5) ——— and GARBER, R. J.
1927. BREEDING CROP PLANTS. Ed. 2; 438 p., illus. New York.
- (6) KVAKAN, P.
1924. THE INHERITANCE OF BROWN ALEURONE IN MAIZE. N. Y. Cornell Agr. Expt. Sta. Mem. 83, 22 p.
- (7) LINDSTROM, E. W.
1923. GENETICAL RESEARCH WITH MAIZE. Genetics 5: [327]-356, illus.
- (8) OWEN, F. V.
1928. CALCULATING LINKAGE INTENSITIES BY PRODUCT MOMENT CORRELATION. Genetics 13: [80]-110.

TOXICITY OF SULPHUR TO SPORES OF SCLEROTINIA CINEREA AS AFFECTED BY THE PRESENCE OF PENTATHIONIC AND OTHER SULPHUR ACIDS¹

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INTRODUCTION

The studies on sulphur reported in the original paper by Young (8)² were summarized as follows: (1) Particulate sulphur is more toxic than the more coarsely ground sulphur; (2) the toxic factor is formed by the action of water and oxygen; (3) this toxic factor is formed only in the acid range and is an acid itself, pentathionic acid; (4) the gases of sulphur are not toxic in quantities present.

Since that work was published many changes have been made in commercial dust formulas. The alkaline type of fluffer has been largely discarded, and the sulphur dusts have been made finer by using a guaranteed 300-mesh instead of a 200-mesh formerly used. These new dusts are much more effective, and consequently the dust method of applying fungicides is gaining ground. The above work has also led to the development of some promising wettable sulphur sprays.

In spite of all these improvements sulphur dusts remain somewhat ineffective under some of the severe conditions for control. Either the dusts are not toxic enough, or they wash off. These problems remain unsolved, and until further progress is made a sulphur dust that will be universally effective for the control of certain fungous diseases at least must remain lacking. It was thought advisable therefore to continue the study of the toxic property of sulphur and especially to determine the effect of the various factors, such as reaction, oxidation, and general weathering, upon the yield of this fungicidal property.

Considerable discussion has arisen about the chemical nature of the exact toxic factor. A few papers have been published, some of which confirm, while others question, the toxicity of pentathionic acid. Tisdale (6) found that pentathionic acid is a toxic factor of sulphur. Lee and Martin (4), assuming that the toxic property of sulphur is an oxidation product, added an oxidizing agent and produced a sulphur dust which was many times more effective than sulphur alone. They have protected their product by a patent. Roach and Glynne (5) tested a great many acids and salts of sulphur and tentatively concluded that the toxicity of sulphur is due to thiosulphuric acid. An examination of their tables shows that pentathionic acid is quite toxic. They stated also that many of their solutions were cloudy, an indication that colloidal sulphur, and consequently pentathionic acid, were present. Young and Williams (9) confirmed the previous work by Young (8) and further showed that ordinary forms of sulphur have pentathionic acid associated with them.

An opposing view of the toxic factor of sulphur is held by Barker and his associates (1). He stated in his report that no pentathionic

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²Reference is made by number (italic) to "Literature cited," p. 962.

acid could be found on sulphur. He did not give the tests used, but probably did not use the same as were used in this laboratory, or he would have obtained a positive test. He attributed the toxic action of sulphur to particulate sulphur. Later, in his annual report (2), he concluded that the toxic action is due to hydrogen sulphide, this being formed by the action of the leaf on the sulphur particle.

The aim of the sulphur work carried on in this laboratory during the past few years has been to develop an effective dusting sulphur. The work has been divided into three parts: (1) The chemistry of sulphur and its toxic factor or factors; (2) toxicity tests; and (3) factors affecting the adhesive properties of sulphur. Work on the chemistry phase has been published by Williams and Young (7), and a more extended reference will be made to it later. The present paper is a report on the second phase, toxicity, and the tests include the products made by Williams and Young.

METHODS

The tests were all made on germinating spores of *Sclerotinia cinerea*. This fungus was selected because of the sensitiveness of its spores to the toxic action of sulphur and their general adaptability to this kind of work. Germination of the spores was not greatly influenced by a hydrogen-ion concentration range between pH 2.2 and 6.0.

The spores which were used for the toxicity tests were scraped from the fruiting area of an 8-day culture and wetted with a little water in a watch glass to make a heavy spore suspension. A drop of the solution to be tested was placed on a glass slide, and spores from the heavy suspension were transferred on a dry glass rod to this drop. In each case a single transfer gave a uniform spore suspension. Equal parts of this drop were spread in a smear on each of four cover slips. These were then inverted over special cells for the germination period. The cells used were cut from a 16-mm. bore glass tubing and placed in Petri dishes in the bottom of which had been fitted a double layer of filter paper with holes cut out for placement of rings. The filter papers were wet to insure a constant and favorable moisture content. After the cover slips were laid on the rings, the Petri dishes were closed and incubated at 24° C. for 16 hours.

The Van Tieghem cell was used in some preliminary trials and was found unsatisfactory. The modified cells described above were uneven on the ends, and conditions such as high vapor pressure and collection of carbon dioxide were eliminated. The hanging-drop cultures gave uneven germination and were consequently discarded.

At the end of the germination period the cover slips were placed smear side up, and counts of the individual spores were made under low power. A spore with no germ tube or with a short dead germ tube was considered not germinated.

The acids of sulphur tested were those isolated by Williams and Young (7). In their work they give tests for and reactions of different compounds associated with sulphur. They include a complete list of reagents and methods of isolation of the various sulphur acids.

There have been two methods used in expressing concentration of these acids. Young and Williams (9) expressed the acid present in percentage. Roach and Glynne (5) developed for their own use a scheme in which the relative amount of sulphur designated by S is

the measure of the dilution of the acids used in toxicity tests. Neither of these expresses the concentration clearly. Different acids of the same concentration have different percentage values. There also seems to be no justification for the use of the symbol S, since there is no confirmed evidence that the atom of sulphur is toxic. Even though the different acids do dissociate into ions containing different amounts of sulphur, these ions apparently act as such, irrespective of the amount of sulphur. It does not seem advisable, therefore, to dilute a solution containing the S_5O_6 = five times to make it comparable to a solution containing the SO_4 = when used for toxicity tests. All the acids used in this work are divalent, and the expression will be given according to their hydrogen-ion concentration, e. g., 0.01 N solution contains the same number of hydrogen ions and the same number of sulphur-containing ions irrespective of the acid in solution.

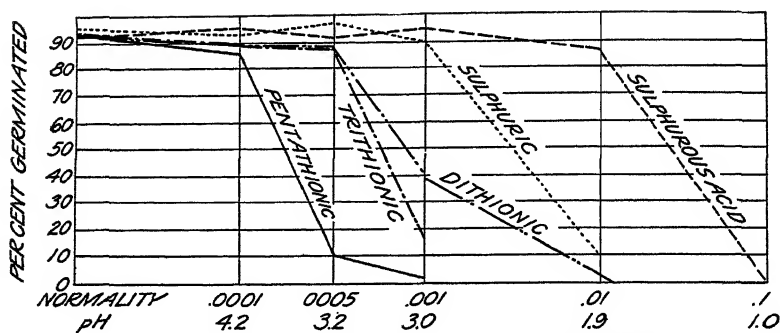
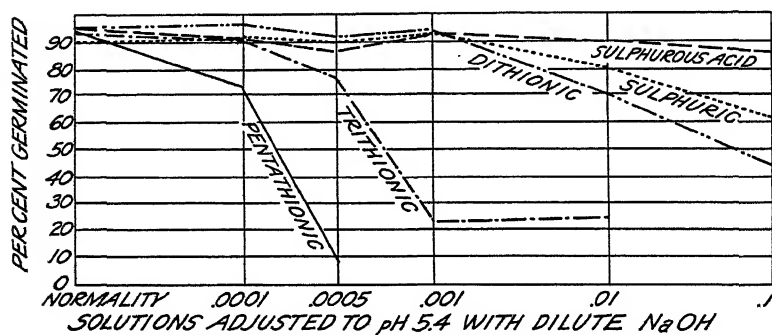
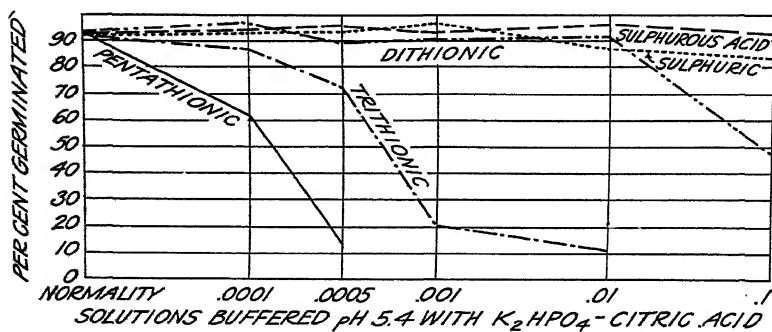
TOXICITY TESTS OF ACIDS OF SULPHUR

The acids tested were sulphuric, sulphurous, dithionic, trithionic, and a mixture of tetrathionic and pentathionic. It was impossible to separate the two last mentioned, and throughout the paper these polythionic acids are called pentathionic, but the contamination with tetrathionic acid is recognized. An attempt was also made to use thiosulphuric acid, but this was found to be impossible under the conditions of the experiments because of its instability. It can not exist on the sulphur particle since it reacts immediately with sulphuric acid to form pentathionic acid. The reaction is given by Freundlich (3) as follows: $H_2SO_4 + 5H_2S_2O_3 \rightarrow 2H_2S_5O_6 + 3H_2O$. Sulphuric acid is normally associated with sulphur, and this precludes the existence of thiosulphuric acid. Roach and Glynne (5) state that their solutions containing thiosulphuric acid up to dilutions of 0.001 N and 0.0005 N were turbid. This would indicate that colloidal sulphur and relatively large quantities of pentathionic acid were present. When such solutions were tested in this laboratory they gave positive tests for pentathionic acid. Inasmuch as their solution, believed to contain thiosulphuric acid, was diluted only twice (according to their scheme of dilution), and their known solution of pentathionic acid was diluted five times, it is only natural that the first solution was more toxic, even though the toxicity of both was due to pentathionic acid.

Thirty different tests were made for each acid. These were made at the same time in double duplicate series. In solutions unaltered, except for dilution, the hydrogen ion is an important factor in its effect upon germination. This effect was eliminated in a second series by the addition of dilute sodium hydroxide. To avoid a possible change in the reaction due to spore germination, a third series was buffered at pH 5.4 with K_2HPO_4 -citric-acid solution. In an effort to throw some light on the action of the S_5O_6 in neutral solutions, the acids in the fourth and fifth series were neutralized, and a buffer was added to the fifth series.

The results of toxicity tests are shown in Figures 1 to 5.

Dilute solutions, such as were used in this work, might be expected to be completely ionized. Consequently all these acids of the same normally have the same number of anions and cations, and the only variable factor is the composition of the anion. It is true that a

FIGURE 1.—Toxicity of acids of sulphur to spores of *Sclerotinia cinerea*FIGURE 2.—Toxicity of acids of sulphur (altered to pH 5.4) to spores of *Sclerotinia cinerea*FIGURE 3.—Toxicity of acids of sulphur (buffered at pH 5.4) to spores of *Sclerotinia cinerea*

variation in the hydrogen-ion concentration will vary the percentage of germination. This is shown by the results given in Figure 1. But when this is kept constant any variation in toxicity must be attributed to the anion. The results of toxicity tests (figs. 2 and 3) show clearly the toxicity of the $S_5O_6=$, all other factors being constant or regulated. Dithionic acid is nontoxic and the trithionic acid

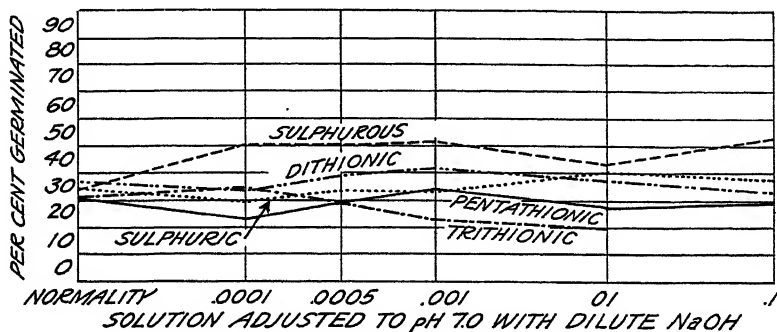


FIGURE 4.—Toxicity of acids of sulphur (altered to pH 7) to spores of *Sclerotinia cinerea*.

solution only slightly so, probably owing to the pentathionic acid unavoidably present. The results given in Figures 4 and 5 show that none of the sulphur-containing ions is toxic in neutral solutions.

TOXICITY TESTS OF SULPHUR FILTRATES

After having ascertained the fact that of all the common acids of sulphur only pentathionic acid was toxic, it was thought important to

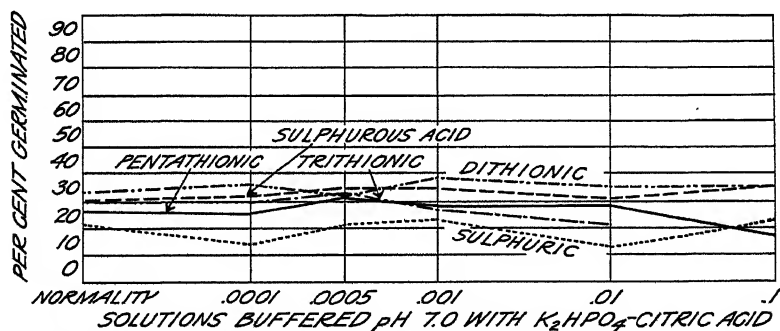


FIGURE 5.—Toxicity of acids of sulphur (buffered at pH 7) to spores of *Sclerotinia cinerea*.

make a study of the acids found directly on or associated with the sulphur particle. This phase of the work was done with the filtrates of ordinary ground sulphur and flowers of sulphur. The amount of acids obtained in such filtrates depends, in a large measure, upon the extent of wetting of the sulphur. This wetting can be accomplished most easily by triturating sulphur with small amounts of water until a thick paste is formed. Ordinarily, 1 c. c. of water to 1 gm. of sulphur is sufficient. The filtrate was then obtained by using a small amount of suction.

In the analysis of this filtrate, although it was early shown that only sulphuric and pentathionic acids and traces of sulphurous acid were present, Williams and Young (7) deemed it necessary to eliminate all other acids from their suspension as well as to prove that these acids were present. Their analysis showed on the average that the acids in the filtrates from ground and flowers of sulphur were, respectively, 7.5 per cent and 12.5 per cent pentathionic acid and 92.5 per cent and 87.5 per cent sulphuric acid. Both dithionic and trithionic acids were absent. In their work 300-mesh ground sulphur and flowers of sulphur were used. Analyses of various commercial sulphur dusts were made. The total acidity of the filtrates from the different sulphur dusts varied somewhat, and the amount of pentathionic acid varied greatly. The results of the analyses are given in Table 1.

TABLE 1.—Analysis of water filtrates from sulphur

Sulphur used	Total acidity	Acidity minus that due to H_2SO_4 *	Acidity due to pentathionic acid
	Per cent	Per cent	Per cent
Flowers of sulphur.....	0.00222	0.000279	12.6
National sulphur.....	.00232	.000181	7.8
Commercial A.....	.00214	.000158	6.0
Commercial B.....	.00330	.000125	3.8

* Traces of sulphurous acid were present.

The toxicity of the filtrates from the same sulphurs was tested as described earlier, equal concentrations of sulphuric acid being used as checks. The results are shown in Figure 6.

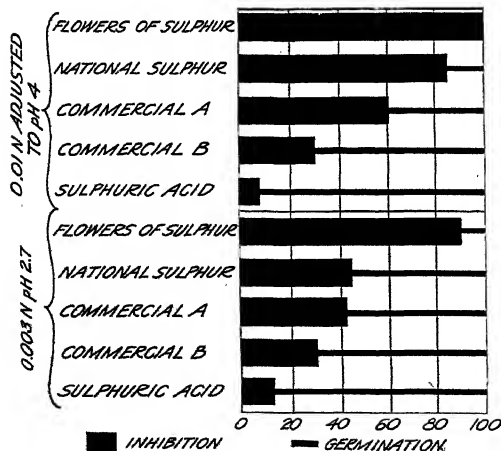


FIGURE 6.—Toxicity of filtrates of various commercial sulphur dusts, in comparison with sulphuric acid of the same concentration, to spores of *Sclerotinia cinerea*

primary tests, and in both cases no particulate sulphur passed through with the filtrate.

A definite amount of sulphur was suspended in different amounts of water by thorough shaking. These suspensions were then used in toxicity tests. (Fig. 7.) Three hundred-mesh ground sulphur was used in all the tests unless otherwise stated. Sulphur suspensions are shown to be toxic to spores of *Sclerotinia cinerea*.

All the filtrates which contained pentathionic acid were toxic and those with the greatest amount of pentathionic acid were the most toxic. In the checks of the same acidity, due, however, to sulphuric acid alone, germination was not inhibited.

No uncombined sulphur was present in the filtrates, and so the possibility of an increase in pentathionic acid was avoided. The Berkfeld filter was compared with extra fine filter paper in preliminary

TOXICITY TESTS OF SULPHUR SUSPENSIONS AND ACID SOLUTIONS WITH THE PENTATHIONIC ACID DESTROYED

It is well known that strong acids and strong alkalis destroy pentathionic acid. A second proof of the toxicity of pentathionic acid was made possible by this characteristic reaction. When ordinary sulphur is treated with concentrated nitric acid or strong ammonium hydroxide and then washed, acid-free sulphur is obtained. It remains acid free when kept in an oxygen-free medium or in contact with one of the reagents. Pentathionic acid solutions with definite toxic properties were treated with dilute sodium hydroxide, dilute ammonium hydroxide, and the strong reagents mentioned above.

The solutions, including checks, were adjusted to pH 5.0 with the proper reagents. These solutions were then tested for toxicity. The results are shown in Figure 8.

Either strong ammonium hydroxide or nitric acid renders a solution of pentathionic acid relatively nontoxic. The solutions tested were not freed from salts formed by the reactions of the reagents added, which in some cases, as indicated in the checks, were of such concentration that the germination was reduced by them.

Heavy suspensions of ground sulphur and flowers of sulphur were treated in the same way, and then filtrates were taken for toxicity

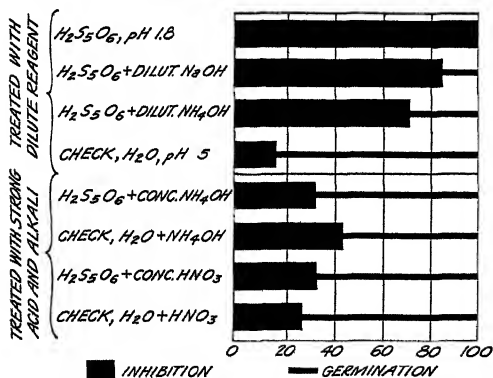


FIGURE 8.—Destruction of pentathionic acid with strong ammonium hydroxide and with nitric acid; tested by the toxicity of treated solutions, compared with untreated solutions of pentathionic acid, to spores of *Sclerotinia cinerea*

particle into the solution. Fifty grams of sulphur was washed in 100 c. c. of water by vigorous shaking in a flask, and then the wash was decanted off and saved for chemical and toxicity tests. Another 100 c. c. of water was then added, and the washing continued in this way until the wash was found to be acid free.

Most of the acid was removed in the first wash. By the third or fourth wash practically all of the acid was removed. The results of toxicity tests are shown in Figure 10.

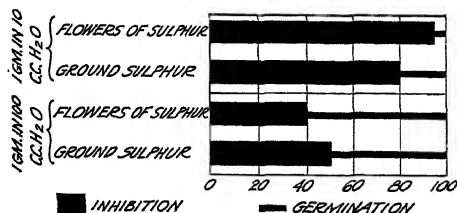


FIGURE 7.—Toxicity of sulphur suspensions (in water) to spores of *Sclerotinia cinerea*

tests. The results are summarized in Figure 9.

The results indicate the effect of these reagents on adsorbed pentathionic acid. Here, as in the experiment with the solutions, the toxicity was largely lost when the pentathionic acid was destroyed.

Spores in sulphur suspensions did not germinate, even though they were not in contact with the sulphur particle. It was thus evident that some of the pentathionic acid washed off the sulphur

The third and later washes from the flowers of sulphur and the fourth and later washes from ground sulphur were not toxic. A comparison with chemical tests given above shows that only those solutions containing pentathionic acid were toxic.

TOXICITY TESTS OF SULPHUR IN OXYGEN-FREE CHAMBERS

In all of the toxicity tests in the preceding experiments with acid-free sulphur only filtrates were used.

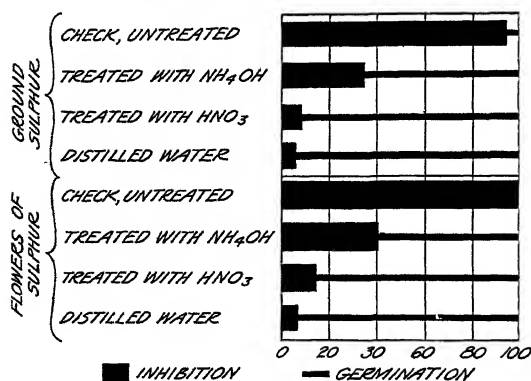


FIGURE 9.—Toxicity to spores of *Sclerotinia cinerea* of filtrates from sulphur which had been treated with strong ammonium hydroxide or with nitric acid to remove pentathionic acid

ber. The spore germination was tested in a suspension of acid-free sulphur in oxygen-free water in an air-tight chamber. The air was then drawn from the chamber by suction while the incoming air was passed through a pyrogallic-acid solution to remove the oxygen. The results are shown in Figure 11.

The water contained enough oxygen to permit 50 per cent of the spores to start germination. The acid-free sulphur had no apparent effect on the germination. Spores and germ tubes were not killed by contact with the sulphur particles, whereas there was no germination in a like toxicity test of ordinary sulphur. This shows that sulphur itself is not toxic but becomes so when oxidized.

THE OXIDATION OF ACID-FREE SULPHUR

Preliminary experiments showed that a suspension of acid-free sulphur became so toxic during the 16-hour germination period that germination was stopped. In order to determine the amount of

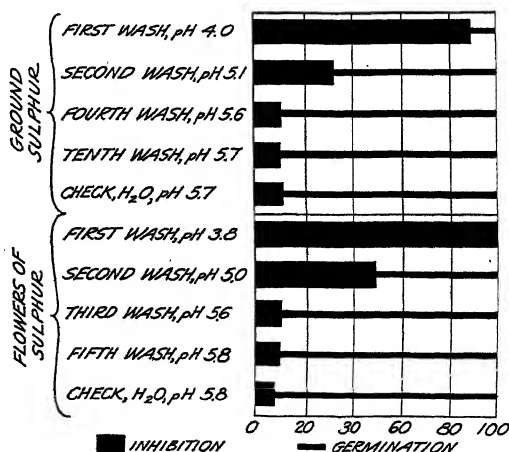


FIGURE 10.—Removal of the toxic factor from sulphur by washing with water, as expressed by the toxicity of the successive washes to spores of *Sclerotinia cinerea*

oxygen used and the rate of oxidation, four 10-gm. samples of acid-free sulphur, prepared by the ammonium-hydroxide and the nitric-acid treatment respectively, were spread on each of two series of four large glass plates. The sulphur was then moistened and exposed to the air for a period of time.

Filtrates were taken from other samples of the same acid-free sulphur and used as checks in toxicity tests. At convenient intervals the sulphur was taken from the plates and filtrates were prepared. The filtering removed all the sulphur, and so any acidity or toxicity which the filtrate possessed would be the result of the oxidation of the sulphur during the period that the sulphur was exposed to the air. These filtrates were then used in toxicity tests. (Fig. 12.)

The tests show that after eight hours of aeration the sulphur had regained its normal toxicity. Even after three or four hours the sulphur was very toxic. The

results of this experiment and the ones preceding it indicate that the toxic factor is an oxidation product of sulphur, and that oxygen is necessary for its occurrence.

THE EFFECT OF WEATHERING ON THE TOXICITY OF SULPHUR

It is well known that sulphur hydrolyzes rapidly at high temperatures (3). One of the end products of the hydrolysis and oxidation of sulphur is pentathionic acid; another is sulphuric acid. When kept at a high temperature a sulphur-lime suspension becomes less alkaline, due to the formation of insoluble and neutral salts by the reactions of sulphur acids, water, carbon dioxide, and oxygen with the calcium hydroxide. Any condition which favors these reactions causes a sulphur-lime suspension to become acid, or at least less alkaline. These conditions are present in weathering processes.

An attempt was made to expose different sulphur suspensions to conditions that would approximate weathering. Five hundred grams

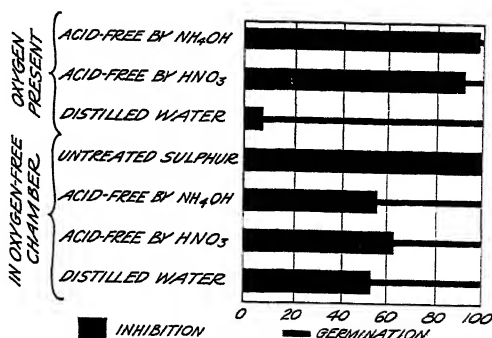


FIGURE 11.—Importance of oxygen in the production of the toxic factor in acid-free sulphur; tests made with spores of *Sclerotinia cinerea* in an oxygen-free chamber in comparison with similar tests made in the presence of oxygen

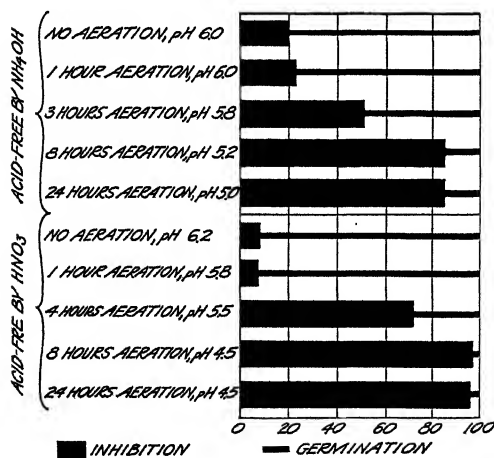


FIGURE 12.—Effect of aeration on the production of the toxic factor in acid-free sulphur, expressed by the toxicity of filtrates of aerated sulphur to spores of *Sclerotinia cinerea*

of acid-free sulphur, sulphur plus a trace of lime, and sulphur-lime (85-15) mixtures were made up, and each was divided into 50-gm. samples. Each sample was put in a flask and well mixed with 50 c. c. water. This suspension was then poured into a large open dish. Duplicate samples of each suspension were exposed to sunlight, heavy shade, laboratory conditions, and at a temperature of 80° C. One sample of each suspension was kept as a check in a closed flask. The other sample was titrated to determine the initial acidity of the suspension.

Each day the water that had evaporated was replaced. At the end of four days the suspensions were placed in flasks and mixed thoroughly. Two c. c. of each suspension were removed for toxicity tests. The suspensions were then titrated with dilute acid or alkali.

The suspensions for toxicity tests were filtered, but no attempt was made to free the filtrates of soluble salts. Therefore, the germination tests were not indicative of the amount of pentathionic acid formed. The results of change in the acidity are recorded in Table 2.

TABLE 2.—*The effect of weathering on the acidity of various sulphurs and sulphur mixtures*

Sulphur	Initial acidity	Treatment	Increased acidity
Acid-free sulphur.....	0.00006	(Sunlight.....	0.01052
		Heavy shade.....	.00305
		Laboratory.....	.00330
		Heat; 80° C.....	.01870
		Check flask.....	.00021
Ordinary sulphur.....	.00405	(Sunlight.....	.00198
		Heavy shade.....	.00119
		Laboratory.....	.00055
		Heat; 80° C.....	.00544
		Check flask.....	.00013
Sulphur plus lime.....	-.00069	(Sunlight.....	.00252
		Heavy shade.....	.00034
		Laboratory.....	.00145
		Heat; 80° C.....	.00028
		Check flask.....	.00165
Sulphur-lime; 85-15 mixture.....	-5.71900	(Sunlight.....	.37700
		Heavy shade.....	.14850
		Laboratory.....	.02200
		Heat; 80° C.....	2.52100
		Check flask.....	-.00078

There was only a slight increase in acidity in the ordinary sulphur. The mixture was hardly wet at all, and because of the formation of a film practically no evaporation took place. About the same condition was present in the neutral sulphur-lime mixtures. In the acid-free sulphur and the sulphur-lime (85-15) mixtures the increase in acidity was definite in all exposed samples. They were wet to form a smooth paste. Evaporation was so great that 50 c. c. of water was added each day to all but the checks in the flasks. A conspicuous layer of white crystals formed on the surface of the sulphur-lime (85-15) mixture. These crystals were insoluble in water. By far the greatest increase in acidity occurred at the high temperature.

The results of this experiment indicate that water is necessary for the formation of acids on sulphur, and that high temperatures are favorable to the reaction.

FIELD TESTS WITH OXIDIZED SULPHUR

Since the ultimate goal of this work is to produce a dusting sulphur that has fungicidal properties equal to sprays, it seemed advisable to try some of the known sulphur-oxidizing agents. Lee and Martin (4) were successful in increasing the toxicity of sulphur by the use of potassium permanganate and nitric acid. In previous experiments the writers have used manganese dioxide. These three oxidizing agents were used in field tests for the control of apple scab, *Venturia inaequalis*, in connection with a number of other experimental dusts. The permanganate dusts were 1 per cent and 2½ per cent. Manganar, which employs manganese dioxide as the oxidizing agent, was used at the rate of 4 per cent for preblossom application and 10 per cent during the postblossom periods. The 300-mesh ground sulphur was used for all dusts except the commercial dusting sulphur. The dust applications were first made in accordance with the timing of the discharge of the spores of the apple scab fungus and, later, ahead of heavy rain periods. In all, seven applications were made. The experiment was conducted in two orchards, one in southern Ohio on Rome Beauty, the other in northern Ohio on Stayman Winesap. The table does not include the results from the southern orchard, since all dusts used were effective. Conditions were not favorable for scab development and infection was not severe. In northern Ohio infection was severe, especially during June and July. The results are given in Table 3.

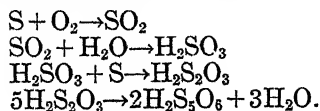
TABLE 3.—Results of field tests with oxidized sulphurs

Material	Total fruit counted	Moderate scabbed	Severe scabbed	Total scabbed
	Number	Number	Number	Per cent
Sulphur+KMnO ₄ (2½ per cent).....	602	22	15	6.1
Sulphur+KMnO ₄ (1 per cent).....	726	61	22	11.4
Sulphur+HNO ₃ (¼ per cent).....	655	55	40	14.3
Sulphur+Manganar (4 per cent).....	675	32	17	7.3
Pure 300-mesh sulphur.....	1,262	196	106	23.9
Commercial dusting sulphur.....	655	116	29	22.1
Check.....				98.5

The results show a marked increase in effectiveness in controlling apple scab. The permanganate plots showed rather severe leaf injury from both leaf drop and scald. This injury came as soon as arsenate of lead was added to the dust and was expected. It is doubtful if the two can be used together in a dust. Manganese arsenate-sulphur mixture gave some leaf injury in one test only, and this was not severe.

DISCUSSION

The results of this work confirm the main point established by Young (8) that the toxic factor of sulphur is pentathionic acid. It seems conclusively established that this factor is an oxidation product of sulphur. The exact manner in which it is formed is at present not definitely known, though it is reasonable to expect that the theory and reactions given by Williams and Young (7) are correct. They state that it is probably formed as follows:



Sulphur dioxide is present only in very small quantities and is considered by them a transitory product in the oxidation of sulphur.

The manner in which pentathionic acid is toxic has not been definitely studied. The above results indicate plainly that the $S_5O_6=$ is at least directly or indirectly responsible. All factors enhancing its production increase toxicity. Whether this ion breaks down in the presence of leaf tissue or germinating spores with the production of other toxic substances is as yet undetermined. The writers have been able to detect hydrogen sulphide on ordinary forms of sulphur when the latter is on leaves or in contact with spores. The theory that hydrogen sulphide is produced by the action of fungus spores on the sulphur particle and is the toxic factor seems untenable, since filtrates from sulphur suspensions contain no particulate sulphur and yet are toxic.

SUMMARY

Pentathionic acid was the only one of the acids tested which showed a marked degree of toxicity. Sulphuric, sulphurous, dithionic, and trithionic acids were only slightly, if at all, toxic.

Sulphur filtrates secured from wetted sulphur contained pentathionic acid and were toxic.

Sulphur that was freed of pentathionic acid, either by strong alkali or acid, or by washing, was not toxic.

Acid-free sulphur which was aerated eight hours regained its normal toxicity. Qualitative tests for pentathionic acid were positive.

Sulphur-lime mixtures when exposed to air, light, and heat in the presence of moisture tended to become less alkaline. Closed checks in the laboratory did not change in reaction.

Field tests confirmed the laboratory results, inasmuch as sulphur, to which an oxidizing agent was added, was more effective than ordinary sulphur in the control of the apple-scab disease.

LITERATURE CITED

- (1) ANONYMOUS.
1926. DISCUSSION ON "THE FUNGICIDAL ACTION OF SULPHUR." *Ann. Appl. Biol.* 13: 308-318.
- (2) BARKER, B. P. T.
1927. INVESTIGATIONS ON THE FUNGICIDAL ACTION OF SULPHUR. PROGRESS REPORT. Univ. Bristol, Agr. and Hort. Research Sta. *Ann. Rpt.* 1927:72-80.
- (3) FREUNDLICH, H.
[1926]. COLLOID AND CAPILLARY CHEMISTRY. Transl. from German ed. 3 by H. S. Hatfield. 883 p., illus. London.
- (4) LEE, H. A., and MARTIN, J. P.
1927. THE DEVELOPMENT OF MORE EFFECTIVE DUST FUNGICIDES BY ADDING OXIDIZING AGENTS TO SULPHUR. *Science* (n. s.) 66: 178.
- (5) ROACH, W. A., and GLYNNE, M. D.
1928. THE TOXICITY OF CERTAIN SULPHUR COMPOUNDS TO SYNCHYTRIUM ENDOBIOTICUM, THE FUNGUS CAUSING WART DISEASE OF POTATOES. *Ann. Appl. Biol.* 15: 168-190, illus.
- (6) TISDALE, L. E.
1925. COLLOIDAL SULPHUR: PREPARATION AND TOXICITY. *Ann. Missouri Bot. Gard.* 12: 381-418, illus.
- (7) WILLIAMS, R. C., and YOUNG, H. C.
1929. THE TOXIC PROPERTY OF SULFUR: CHEMISTRY IN RELATION TO TOXIC FACTORS. *Indus. and Engin. Chem.* 21: 359-362.
- (8) YOUNG, H. C.
1922. THE TOXIC PROPERTY OF SULPHUR. *Ann. Missouri Bot. Gard.* 9: 403-435, illus.
- (9) ——— and WILLIAMS, R. C.
1928. PENTATHIONIC ACID, THE FUNGICIDAL FACTOR OF SULPHUR. *Science* (n. s.) 67: 19-20.

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BACTERIAL STREAK DISEASE OF SORGHUMS¹

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INTRODUCTION

Specimens of the disease here designated as bacterial streak disease were first seen by the writer on leaves of a feterita-milo hybrid (F. C. I.³ 8926) sent by H. N. Vinall from Chillicothe, Tex., in 1921. The disease was found at Hays, Colby, and Manhattan, Kans., in 1924 on a large number of sorghum varieties, some of which were badly streaked with the disease from the lowest to the uppermost leaves. Specimens of the disease also have been sent in from Woodward, Okla., and Glendive and Huntley, Mont., and have been collected again recently at Chillicothe, Tex.

SYMPTOMS

The bacterial streak disease is characterized by dark reddish-brown streaks on the leaves of *Holcus sorghum* and *H. halepensis*. (Pl. 1; figs. 1, 2, and 3, B.)

The youngest lesions (fig. 2, A) are narrow water-soaked streaks 2 to 3 mm. broad and 2 to 15 cm. or more long. They show no red coloring and occur on plants from the seedling stage, with only two leaves, to those with heads nearing maturity.

Narrow red-brown margins or blotches of color soon appear in the water-soaked streaks (fig. 2, B) and in a few days the red color is continuous throughout the lesions. Older lesions are no longer translucent. (Fig. 2, C.) At intervals these red streaks broaden into elongated oval spots with tan centers and narrow red margins. (Pl. 1, B, and fig. 1.) These lesions, when numerous, coalesce to form long, irregular streaks and blotches covering a part or the whole width of the leaf blade and having more or less dead tissue with narrow dark margins between the reddish-brown streaks. Exudate is abundant (fig. 3, A), standing out on the young lesions as light-yellow beads which dry to thin white or cream-colored scales.

ISOLATIONS AND INOCULATIONS

Isolations were made both by washing the tissue through 12 sterile water blanks and by dipping it in 95 per cent alcohol and then for two minutes in 1:1,000 mercuric chloride and washing. These isolations have repeatedly given the same yellow colonies in either pure or mixed cultures.

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² The writer wishes to express appreciation to A. F. Swanson, assistant agronomist of the Office of Cereal Crops and Diseases, for assistance in making the observations at Hays, Kans., and to J. F. Brewer, chief scientific illustrator of the Office of Mycology and Disease Survey, for making the colored plates and the photographs.

³ F. C. I. indicates a serial number of the Office of Forage Crops and Diseases.

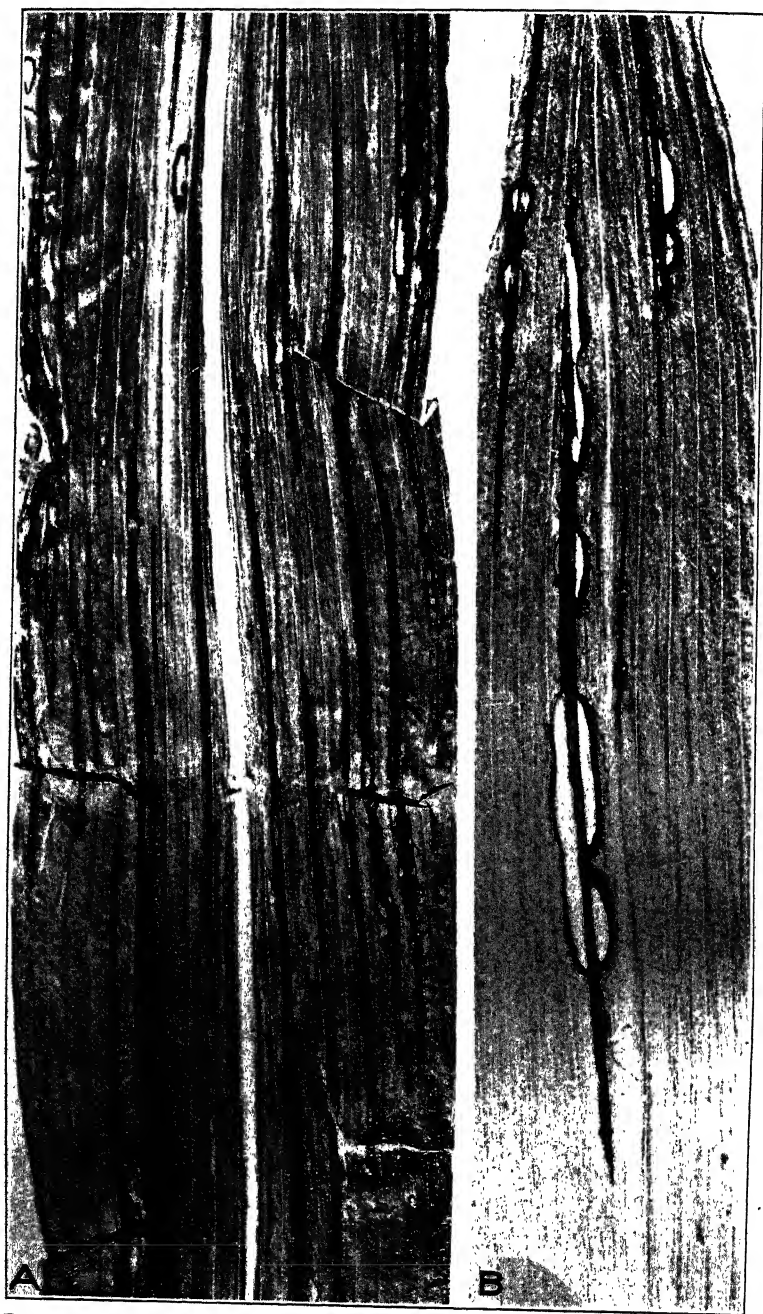


FIGURE 1.—Natural infections on Black Amber sorgho (A) and Wonder kafir (B). Collected at Hays, Kans., August 7, 1924. $\times 1$

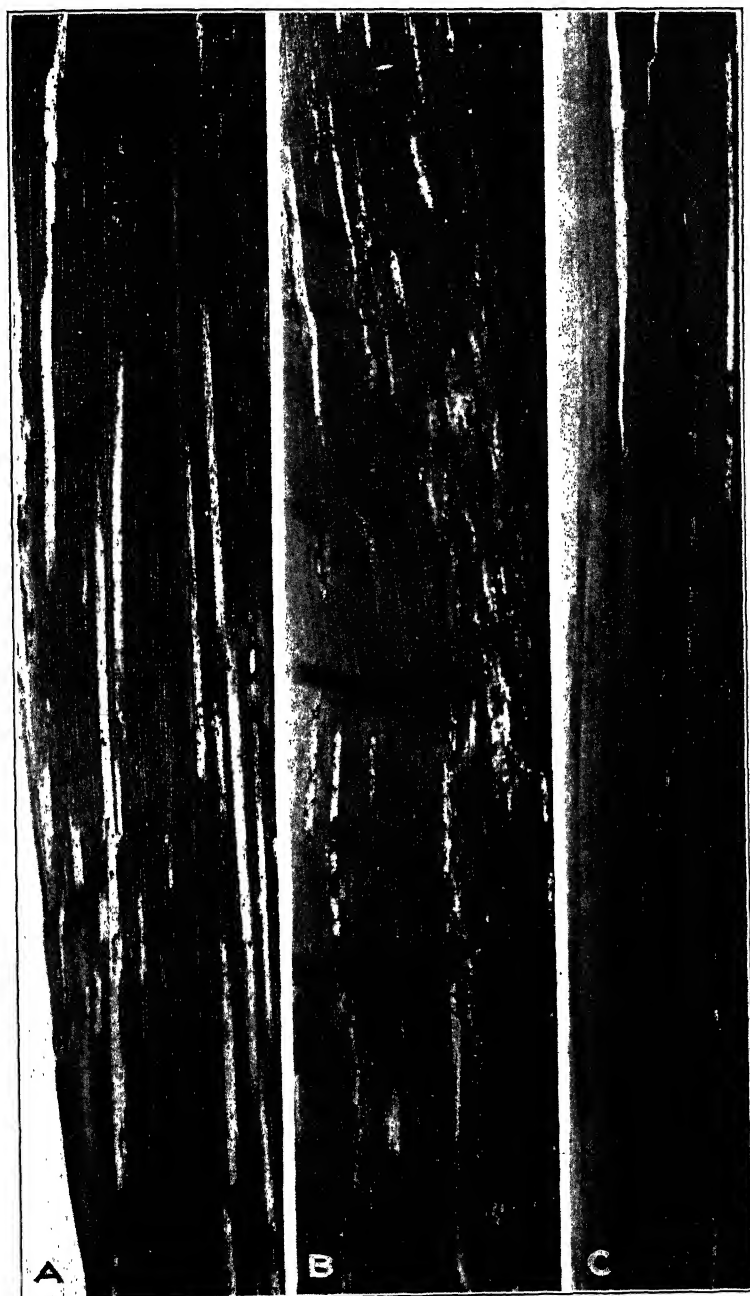


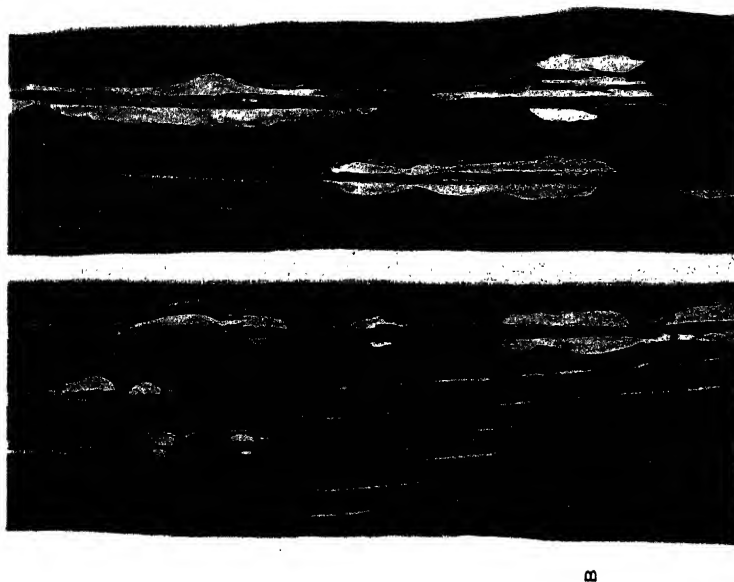
FIGURE 2.—Natural infections of different ages photographed by transmitted light to show different degrees of translucency. Collected at Hays, Kans., 1924. $\times 1$. A, Young translucent lesions on Pink kafir \times milo. B, Slightly older and only partly translucent lesions on Pink kafir \times Dwarf milo. C, Older lesions, which are no longer translucent, on Farr's Dwarf hegari.



FIGURE 3.—A, Natural infection, with abundant exudate scales, on Pink kafir \times milo. Collected at Hays, Kans., August 28, 1924. Photographed September 3, 1924. $\times 4$. B, Natural infection on Johnson grass. Collected at Chillicothe, Tex., August, 1928. $\times 1$

Bacterial Streak Disease of Sorghums

PLATE 1



A, Young translucent lesions of bacterial streak on Pink kafir sorghum collected at Hays, Kans., 1924. X 1. B, Older lesions of bacterial streak on Wonder kafir collected at Hays, Kans., 1924, showing typical elongated oval spots along the streaks. X 1

Of the 60 isolations from typical lesions of this disease, 14 were made from the original material from Chillicothe, Tex., in 1921, 34 from material collected at Hays, Kans., in 1924, and the remainder from lesions collected at Chillicothe, Tex., at Woodward, Okla., at Glendive and Huntley, Mont., and at Rosslyn, Va. Recently the same organism has been isolated from the same type of lesion on Johnson grass collected at Chillicothe, Tex. Yellow colonies developed on all the plates from these lesions. Some of them appeared in one to two days, but the colonies of the parasite developed more slowly, becoming visible only on the third or fourth day and showing the light-yellow color only after they had reached a diameter of about 2 mm.

The persistent appearance of this slow-growing light-yellow organism in large numbers on the plates led to the assumption that it must be the cause of the disease, but proof of its pathogenicity was wanting for some time. Inoculations were made by spraying wounded and unwounded plants with water or broth suspensions from young agar cultures. The inoculated plants, together with the controls sprayed with sterile water, were held in damp chambers for several days after inoculation.

Fifteen inoculations from organisms isolated from the material collected in Texas in 1921, were made on feterita \times milo, feterita, broomcorn, Dwarf kafir, an African sorghum collected by H. L. Shantz, Early Fortune proso (S. Dak. No. 98), and feterita \times Red Amber. Not a single lesion developed on any of the plants. In the field pieces of infected leaf tissue with abundant exudate were tied to healthy leaves, which were pierced with a needle and covered with moist cotton, but this procedure always failed to produce infection.

During the fall and winter of 1924-25 about 40 inoculations were made with isolations from materials collected at Hays, Kans., in 1924. In only 6 of these inoculations were lesions produced on inoculated plants, and none of these artificial infections was more than 1 to 2 mm. broad and 6 to 20 mm. long. Exudate was present on two lesions and from sections under the microscope bacteria streamed in abundance. From three of these lesions typical cultures of the light-yellow organism were reisolated.

While isolations Nos. 72 and 80 in one experiment produced lesions up to 20 mm. long, these same cultures, tested twice afterwards under apparently similar conditions, failed to produce any infections.

In the fall of 1927 the same yellow organism was isolated from young lesions on Dwarf White milo collected at Chillicothe, Tex. This isolation, No. 89, was sprayed on plants of Dwarf White milo growing in temperature tanks at 24°, 32°, and 36° C. No lesions developed on plants growing at 32° or 36°, but on plants growing at 24° 10 small lesions developed. They were translucent, more or less red, 1 mm. wide, and up to 6 mm. in length. The typical yellow organism was reisolated. This same organism used for inoculation in December apparently produced no lesions, but four months later typical red lesions 2 to 10 cm. long were found on these plants. The plants were cut down before the organism was reisolated.

At the suggestion of A. G. Johnson, this same organism was used again for inoculation by spraying the plants after dark and covering them with inverted flower pots. Ten days after inoculation typical

lesions of the disease developed on several plants. The lesions were translucent, red along the margins or throughout, 3 to 4 mm. in diameter, and 10 to 15 cm. long. Pure cultures of the lemon-yellow organism were reisolated from three of these lesions. One of these reisolations again was used for spraying milo plants after dark. Also one set of plants was sprayed about noon and both sets were covered with earthen pots. (Fig. 4, A.) Both the plants inoculated after dark and those inoculated about noon developed a few small lesions 2 to 20 cm. long, from which the typical yellow colonies were reisolated.

The organism isolated from lesions on Johnson grass and sprayed on Dwarf White milo after dark has produced abundant typical infections (fig. 4, B), and the yellow organism has been reisolated and again sprayed on Dwarf Yellow milo. The pathogenicity of the organism thus has been established, but the conditions that limit infection have not been made clear. The only inoculation producing abundant infection was made after dark.

THE CAUSAL ORGANISM

MORPHOLOGY

Grown on beef-peptone agar and other nutrient media, the causal organism which is described technically as *Bacterium holcicola* n. sp. on page 972, is a short rod with rounded ends, arranged singly, in twos, or in threes. Grown on beef-infusion peptone agar and stained with carbol fuchsin, the rods vary in length from 1.05μ to 2.40μ and in diameter from 0.45μ to 0.90μ and average 0.65μ by 1.44μ . Grown on Thaxter's potato-dextrose agar and stained with carbol fuchsin, they vary in length from 1.3μ to 2.4μ and in diameter from 0.65μ to 1.1μ and average 0.84μ by 1.70μ . Capsules are present on beef-peptone agar cultures stained with Casares-Gil flagella stain and with Ribbert capsule stain. (Pl. 2, B.) Stained with the Casares-Gil flagella stain, the organism is shown to be motile on agar by 1 to 2 polar flagella. (Pl. 2, A, B.)

STAINING REACTIONS

Stained by the Hucker modification of the Gram stain, the causal organism is Gram-negative. Stained by the Ziehl-Neelsen method it is not acid-fast. It stains readily with carbol fuchsin and aniline gentian violet.

CULTURAL CHARACTERS

BEEF-INFUSION PEPTONE AGAR SLANT.—Growth is moderate, filiform, flat, glistening, smooth, translucent, light wax yellow,⁴ and butyrous to slightly

⁴ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.

EXPLANATORY LEGEND FOR PLATE 2

A and B.—Smears from 2-day agar slants of *Bacterium holcicola* (isolation No. 89). Stained with Casares Gil's stain, November 15, 1928. $\times 1,400$.

C.—Seventeen-day colonies of *Bact. holcicola* (isolation No. 72) from Yellow milo, on beef-peptone agar, showing secondary growth in the original colonies. Photographed by transmitted light, February 17, 1928. $\times 4$.

D.—Six-day colonies of *Bact. holcicola* (isolation No. 89) on beef-peptone agar. Photographed February 14, 1928, by transmitted light to show amorphous character. $\times 5$.

E.—Six-day colonies of *Bact. holcicola* (isolation No. 89) on Thaxter's potato agar. Photographed February 14, 1928, by reflected light to show smooth surfaces and entire margins. $\times 5$.

F.—Thirteen-day surface and embedded colonies of *Bact. holcicola* (isolation No. 95) on beef gelatin. Photographed February 13, 1928. $\times 2$.

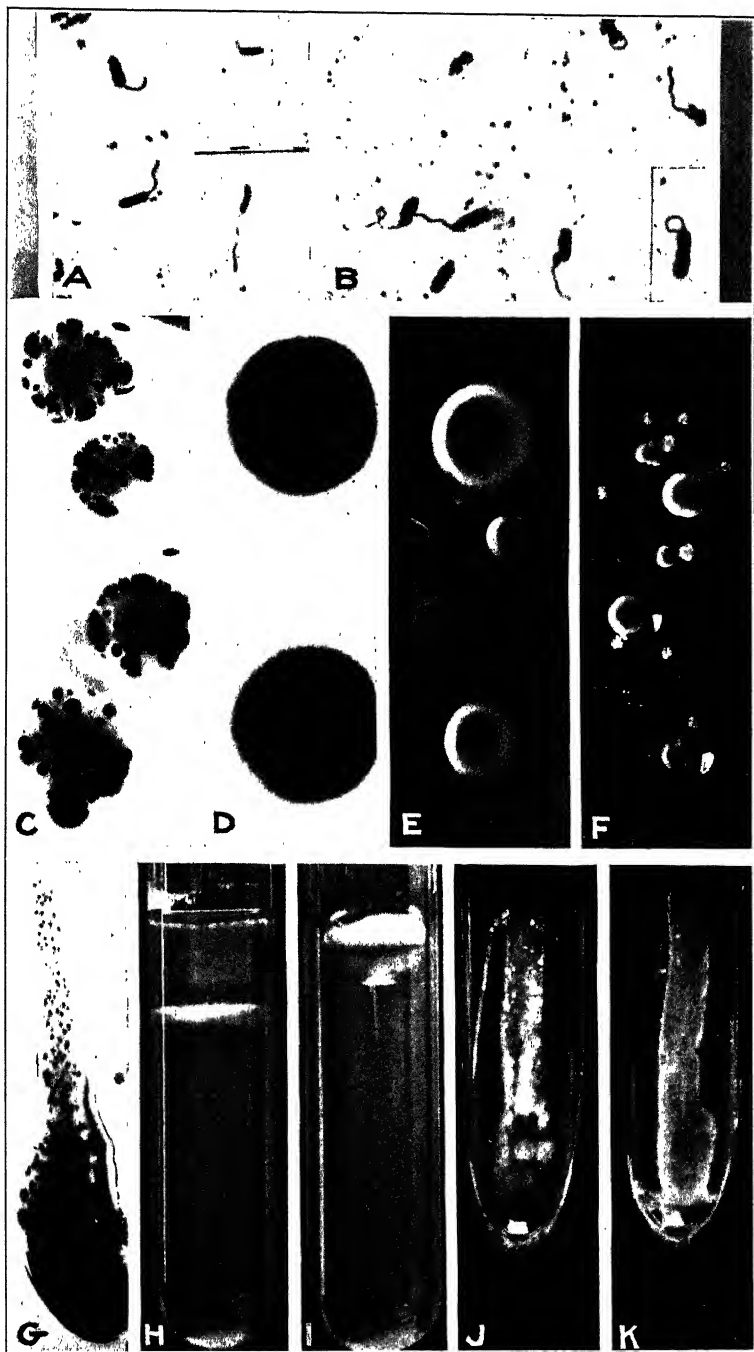
G.—Fourteen-day beef-peptone agar slant of *Bact. holcicola* (isolation No. 80). Photographed February 19, 1928, by transmitted light to show secondary colonies in original slant culture. $\times 2$.

H.—Fourteen-day beef-gelatin stab of *Bact. holcicola*. Photographed February 14, 1928, to show stratiform liquefaction. $\times 1$.

I.—Fourteen-day beef-gelatin stab of *Bact. holcicola* (isolation No. 89). Photographed February 14, 1928, to show napiform liquefaction. $\times 1$.

J.—Twenty-day beef-peptone agar slant of *Bact. holcicola* (isolation No. 72), showing secondary colonies. Photographed March 21, 1928. $\times 1$.

K.—Twenty-day beef-peptone agar slant of *Bact. andropogoni*. Photographed March 21, 1928. $\times 1$.



For explanatory legend see opposite page

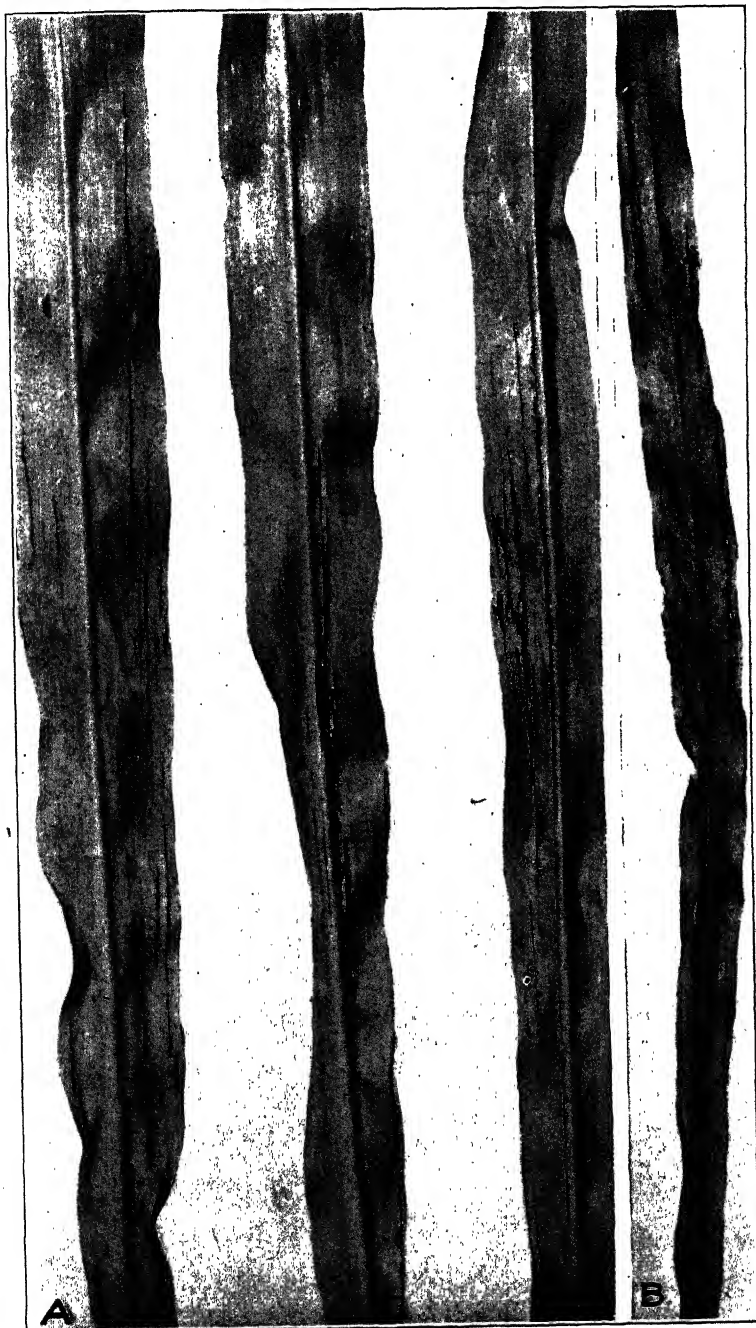


FIGURE 4.—Infections on Dwarf White milo from artificial spray inoculations in the greenhouse at Washington, D. C. A, Inoculated May 7, 1923, with culture No. 89, isolated from Early White milo (C. I. 450), collected at Woodward, Okla., September, 1926. Photographed June 5, 1928. $\times 1$. B, Inoculated November 12 1923, with culture No. 95, isolated from Johnson grass, collected at Chillicothe, Tex. August, 1923. Photographed December 1, 1923. $\times 1$

viscid in old cultures. The medium is unchanged. Secondary colonies begin to show in large numbers as tiny round colonies over the surface of the slant when the culture is about three weeks old. (Pl. 2, G, J.)

BEEF-INFUSION PEPTONE AGAR PLATES.—Colonies become visible in three to four days. They are round, with entire margins, umbonate, glistening, smooth, at first translucent, creamy white but in a few days becoming a light wax yellow and more opaque, butyrous, without internal markings except slight radiate lines. (Pl. 2, D, E.) After about two weeks, when the agar begins to dry, secondary colonies begin to develop along the margins and through the centers of the original colonies. (Pl. 2, C.)

THAXTER'S POTATO-DEXTROSE AGAR SLANTS.—Growth is abundant, raised, filiform to spreading, smooth, and glistening; a deep-cream color occurs along the slant and yellow in the condensation water at its base.

THAXTER'S POTATO-DEXTROSE AGAR PLATES.—Colonies are visible in two to three days. They are at first white to cream colored, umbonate, glistening, round with entire margins, amorphous to slightly radiate by transmitted light; after four to five days they become light yellow (pl. 2, E), and after two to three weeks secondary V-shaped colonies may begin to develop as on beef-infusion agar.

GELATIN STABS.—At room temperature growth is best at the top, and a colony of 2 to 3 mm. in diameter forms in two days. Liquefaction begins on the third day. The surface colony becomes slightly sunken and beneath it is produced a small pit of liquefaction into which the surface growth sinks. Liquefaction is napiform (pl. 2, I) for the first two weeks, after which it becomes stratiform. In three weeks the gelatin is about one-fourth liquefied.

BEEF GELATIN PLATES.—Small colonies appear on the third day. Colonies become bright yellow and more or less lobed around the margins, and about the tenth day they begin to sink into saucer-shaped depressions. (Pl. 2, F.) Heavily seeded plates are entirely liquefied. At the end of about three weeks scattered colonies are 4 to 5 mm. in diameter in depressions 8 to 10 mm. in diameter.

BEEF-INFUSION BOUILLON.—There is a trace of growth in 24 hours and light clouding in 48 hours with a scattered flocculence along one side of the tube from top to bottom. By the third day there forms at the surface a slight ring which is readily broken up into flocculent particles but which increases from day to day until it forms an irregular yellow ring and the clouding becomes moderate. A small quantity of sediment is formed which is viscid on agitation.

COHN'S SOLUTION.—Two cultures showed a trace of clouding in Cohn's solution, pH 5.2, in three to five days, but this gradually disappeared, and at the end of two weeks there was no evidence of any growth. Another test in a medium with a pH 5.3 showed no growth at all. The reaction of this medium is more acid than the minimum for this organism.

FERMI'S SOLUTION.—There is never more than a light clouding and after two months a viscid ring or pellicle strings down into the medium.

USCHINSKY'S SOLUTION.—At pH 6.4, there is a fine flocculent growth along the sides of the tube after eight days. This falls to the bottom and is more or less viscid. After about two months there is a very light clouding of the medium. In another test at pH 6.3 there was no growth at all.

PHYSIOLOGY

TEMPERATURE RELATIONS.—The optimum temperature for growth is about 28° to 30° C.; the maximum, 36° to 37°; and the minimum, 4°. The thermal death point is 51°.

RELATION TO REACTION OF MEDIUM.—Grown in beef-infusion peptone broth, the optimum reaction is pH 7.0 to 7.5, and the limits of growth are pH 5.5 and 9.0.

CHROMOGENESIS.—On nutrient agar the color of this organism is a wax yellow⁵ and on potato-dextrose agar a deep cream along the streak and a wax yellow at the base. On potato the color is more of an empire yellow.

PRODUCTION OF INDOL.—Grown in Dunham's solution and tested with Ehrlich's test, the organism produced no indol, although a culture in which *Bacillus coli* was grown at the same time gave a positive reaction for indol. Grown in tryptophane broth and tested after 2 to 4 and 11 days by the Ehrlich-Böhme, the Goré modification of the Ehrlich-Böhme, and by the Gnezda technic, there was no reaction for indol.

PRODUCTION OF HYDROGEN SULPHIDE.—On lead-acetate agar stabs growth becomes dark brown at the surface and along the stab, indicating the formation of hydrogen sulphide.

⁵ RIDGWAY, R. Op. cit.

RELATION TO OXYGEN.—In fermentation tubes containing sucrose, glucose, lactose, and glycerin there was growth only in the open arm. Shake cultures in beef-infusion peptone agar plus dextrose grew only at the surface. The organism is an aerobe.

MILK.—No curd is formed, and peptonization becomes evident about the sixth day and is completed in about 10 days. The reaction is the same in litmus milk. The color becomes a deeper shade of lavender or slightly more blue than the control and about the tenth day is reddish purple. By the sixteenth day the tubes are colorless. Old milk cultures become gelatinous.

NITRATE REDUCTION.—Three tests with nitrate broth gave no reaction for nitrites, although cultures of oat organisms in the same media gave definite evidence of reduction.

Two tests were made with isolations Nos. 89 and 95 on media recommended by the Society of American Bacteriologists.⁶ The media were as follows: A, Nitrate-peptone broth; B, peptone broth without nitrate; C, a synthetic nitrate medium (KNO_3 , 1 gm.; K_2HPO_4 , 0.5 gm.; CaCl_2 , 0.5 gm.; glucose, 10 gm.; distilled water, 1,000 c. c.); D, peptone broth with 2 parts per million potassium nitrite. No. 89 is an older culture and does not grow so vigorously as No. 95. This would account for slight differences in results with the two strains.

The organism grew well in A, B, and D, but there was only light clouding in the synthetic medium in both tests. In the first set the test for ammonia was made with strips of filter paper moistened with Nessler's solution and suspended over the cultures. A, B, and C were tested for ammonia. Uninoculated controls showed no reaction for ammonia. Cultures in the synthetic medium also showed no reaction for ammonia.

In 10 days both No. 89 and No. 95 showed slight browning of the filter paper in peptone broth. In nitrate broth there was no browning in No. 89 and decided browning of the lower edge in No. 95.

In 19 days in nitrate broth No. 89 showed a trace of browning on the edge of the filter paper and No. 95 turned the paper black for 5 to 6 mm.

In peptone broth the filter paper in both cultures was dark brown on the lower edge.

Tests for nitrites were made with sulphanilic acid and α -naphthylamine.

In the synthetic medium there was never any reaction for nitrites.

In D (peptone broth plus 2 parts per million nitrite) tests for nitrites were made on the first, second, fourth, seventh, thirteenth, and thirty-seventh days. Controls were always a deep-pink or rose color. Up to the thirteenth day the cultures were as deep pink as the controls, but on the thirty-seventh day No. 89 was light pink and No. 95 very light pink.

In the second test growth was the same as in the first. The Thomas test was used for ammonia and sulphanilic acid and α -naphthylamine for nitrites. The results of tests for ammonia in nitrate broth and in peptone broth are shown in Table 1.

TABLE 1.—Results of Thomas tests for ammonia in nitrate broth and in peptone broth, uninoculated (control) and inoculated with *Bacterium holcicola* cultures Nos. 89 and 95, respectively

Days after inoculation	Control or culture No.	Results of color tests for ammonia in—	
		Nitrate broth	Peptone broth
1.....	Control.....	Greenish.....	Trace green.
1.....	89.....	Slightly green.....	No color.
1.....	95.....	No color.....	Do.
3.....	Control.....	Greenish.....	Green.
3.....	89.....	do.....	Less green than control
3.....	95.....	Slightly bluer than control.....	Do.
7.....	Control.....	Slightly green.....	Slightly green.
7.....	89.....	No color.....	No color.
7.....	95.....	do.....	Do.
20.....	Control.....	Trace bluish throughout.....	No color.
20.....	89.....	Deep blue in lower third.....	Deep blue in lower fourth.
20.....	95.....	do.....	Do.

⁶ SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIOLOGICAL TECHNIC. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA, FOR USE WITH THE DESCRIPTIVE CHART OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS . . . 45 p., illus. Geneva, N. Y. 1926.

In synthetic media isolations Nos. 89 and 95 showed no reduction of nitrates on the second, fourth, ninth, and twenty-first days. At no time was there any reaction for ammonia.

Results of tests for nitrites in beef-extract broth plus 2 parts per million potassium nitrite uninoculated (control) and inoculated with Nos. 89 and 95 were as follows: Second and fourth days, all deep pink; ninth day, control and No. 89 deep pink, but No. 95 a lighter pink than control; and twenty-first day, control deep pink, No. 89 light pink, and No. 95 very light pink.

There is no evidence of gas production in fermentation tubes containing cultures in nitrate-peptone broth.

Since the organism produces ammonia in peptone broth without nitrate, grows only slightly in the synthetic medium, and slowly destroys the nitrite in peptone broth containing 2 parts per million nitrite, the test for nitrate reduction is recorded as inconclusive.

HYDROLYSIS OF STARCH.—There is a partly cleared zone 1 cm. broad around the streak on starch-agar plates after growth for 10 days and 2 cm. broad after 19 days.

FERMENTATION.—Tests were made with sucrose, glucose, lactose, and glycerin, using 2 per cent sugar plus 1 per cent peptone in distilled water, 2 per cent sugar plus 0.2 per cent peptone, 2 per cent sugar plus 0.2 per cent peptone plus 0.5 per cent tartrate, 2 per cent sugar plus 0.5 per cent peptone, and 1 per cent sugar plus ammonium phosphate, as recommended in the Manual of Methods.⁷

No gas was formed in fermentation tubes, and when tested with neutral litmus paper at the end of 16 days the reaction was alkaline in all cultures.

In three tests brom-cresol purple, phenol red, and brom-phenol blue were used as indicators in the media. The cultures were alkaline throughout with dextrose, but with sucrose there was a slight production of acid during the first few days, the reaction later becoming alkaline.

In seven tests quantitative colorimetric pH determinations were made. The results are given in Table 2.

TECHNICAL DESCRIPTION

***Bacterium holcicola* n. sp.**

A motile rod with rounded ends and one to two polar flagella; single, in pairs, or occasionally in short chains; average measurements 0.75μ by 1.58μ ; no spores; capsules formed; Gram-negative; not acid fast; stains readily with carbol fuchsin and gentian violet; beef-infusion peptone agar colonies are round, umbonate, glistening, smooth, at first translucent, later more opaque, wax yellow, butyrous, amorphous or with slight radiate markings by transmitted light; gelatin is liquefied slowly; growth in beef-infusion bouillon is moderate and an irregular ring forms at the surface; there is no growth in Cohn's solution (?); slight growth in Uchinsky's solution and light clouding in Fermi's solution and formation of a viscid ring which strings down into the medium; optimum temperature for growth is about 28° to 30° C., maximum 36° to 37° , minimum about 4° ; thermal death point 51° ; optimum reaction for growth is pH 7.0 to 7.5; the limits of growth, pH 5.5 and pH 9.0; indol is not produced; hydrogen sulphide and ammonia are produced; aerobic; milk is cleared without coagulation; nitrate reduction doubtful; hydrolysis of starch moderate; no gas produced from carbon compounds; a slight amount of acid is formed from sucrose; the reaction with glucose, lactose, and glycerin is alkaline. Pathogenic on leaves of varieties of *Holcus sorghum* and *H. halepensis*.

⁷ SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIOLOGICAL TECHNIC. Op. cit.

TABLE 2.—Changes in reaction caused by the growth of *Bacterium holcicola* in culture solutions containing carbon compounds

[Quantitative colorimetric determinations were made]

Date inoculated and medium used	Inoculated with culture No.	Incubation period	Hydrogen-ion concentration of medium containing—							
			Sucrose		Glucose		Lactose		Glycerin	
			Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
Nov. 6, 1924: Peptone, 0.2 per cent; carbon compound, 2 per cent.	71	Days								
		2			6.0	5.8				
		4			6.4	5.8				
		7			6.5					
		9			6.6	5.6				
		11			6.7	5.7				
		14			7.0					
		18			7.1	5.7				
		22			7.1	5.6				
		2			6.3	6.1				
Peptone, 0.2 per cent; carbon compound, 2 per cent; tartrate, 0.5 per cent.	71	4			6.5	6.1				
		7			6.6					
		9			6.7	6.1				
		11			6.7	6.1				
		14			7.0					
		18			7.1	6.1				
		22			7.2	6.1				
		0		5.8		5.6				
		2	6.1	5.9	5.9	5.6				
		3	5.9	5.8	5.9	5.6				
Nov. 26, 1924: Peptone, 0.2 per cent; carbon compound, 2 per cent.	72	5	5.8	5.8	6.0	5.6				
		7	6.1		6.4					
		10	6.4		6.7					
		13	7.0	5.8	6.9	5.5				
		16	7.3		6.9					
		21	7.4	5.9	7.1	5.4				
		31	7.5	6.2	7.6	5.7				
		0				5.8				
		2			5.9					
		3			6.0					
Jan. 3, 1925: Peptone, 0.2 per cent; carbon compound, 2 per cent.	72	4			6.1	5.6				
		6			6.3					
		7			6.3	5.8				
		1				5.9				
		2			6.0					
Jan. 12, 1925: Peptone, 0.2 per cent; carbon compound, 2 per cent.	72	4			6.0	5.7				
		7			6.0	5.7				
		9			6.3	5.7				
		11			6.4					
					6.8					
Feb. 9, 1925: Ammonium phosphate; carbon compound, 1 per cent.	72	0				6.9				
		1			6.8	6.9				
		2			6.8					
		5			6.8					
		7			6.8	6.9				
		8			6.8					
		9			6.7					
		10			6.7					
		12			6.7					
		15			6.7	6.9				
		16			6.7					
	18			6.4						
Nov. 22, 1928: Peptone, 0.5 per cent; carbon compound, 2 per cent.	39	1	6.9	7.1	6.6	6.6	6.8	6.9	7.0	7.0
		2	6.8	7.0	6.6	6.5	6.8	6.8	6.9	7.0
		4	6.6	7.0	6.4	6.5	6.8	6.7	7.1	7.1
		8	6.9	7.1	6.8	6.5	7.2	6.8	7.3	7.0
		11	7.1	7.1	7.0	6.4	7.3	6.7	7.2	7.0
	95	21	7.4	7.1	7.4	6.4	7.6	6.6	7.6	6.9
		1	7.0	7.1	6.6	6.6	6.8	6.9	7.0	7.0
		2	6.8	7.0	6.6	6.5	6.8	6.8	7.0	7.0
		4	6.6	7.0	6.5	6.5	6.9	6.7	7.1	7.1
		8	7.0	7.1	6.8	6.5	7.2	6.8	7.3	7.0
	11	7.2	7.1	7.3	6.4	7.5	6.7	7.4	7.0	
	21	7.6	7.1	7.7	6.4	7.7	6.6	7.6	6.9	

VARIETAL SUSCEPTIBILITY AND MEANS OF DISSEMINATION

The heaviest infection of this disease observed by the writer occurred at Hays, Kans., in 1924.

The varieties listed were grown in a test plot in blocks of 4 to 24 and 48 rows of each variety, each row representing a selection. There were considerable differences in amounts of infection on different plants in the same row, but on the whole they could be listed as follows:

White kafir (C. I.⁸ 342) was the only one that appeared to be practically immune, with only an occasional lesion.

White kafir (C. I. 314) and Leoti Red sorgo also showed very little infection.

Sunrise kafir, Progressive kafir, Barchet kaoliang, Early White milo, Buff durra, and a selection of Red Amber \times feterita all showed traces of spotting on most of the leaves of all of the plants.

Kafir (C. I. 204), Bishop kafir, Reed kafir, Freed sorgo, Dawn kafir, and Pierce kaferita (H. C.⁹ 2523) all showed occasional plants with half of the leaves lightly streaked.

Dwarf feterita, Freed sorgo, Straight-neck milo, Farr's kafir \times feterita, and Tricker sorgo all showed a moderate amount of spotting on 50 to 100 per cent of the leaves of all plants in a row.

Pink kafir \times milo \times feterita, Pink kafir \times milo, Dwarf Yellow milo \times Dwarf hegari, Wonder kafir, Farr's hegari \times milo, Farr's European milo, Farr's Dwarf hegari, and Pink kafir \times Dwarf milo all showed heavier spotting than the other varieties, some plants in each showing fairly heavy spotting on all leaves from the lowest to the uppermost.

There were enough lesions on leaves just below the heads to make it quite possible that the seeds might become infected and transmit the disease. To test this, heads from heavily infected plants were collected and the seed sown at the Arlington Experiment Farm, Rosslyn, Va., in May, 1925. Half of each of three lots of seed was treated with uspulun, 0.25 per cent, for one hour, and then washed; and half of each of four lots with formaldehyde, 1:320, for two hours, and washed. The other half of each lot was untreated. Not a single lesion was found on any of these plants throughout the season.

Several attempts to isolate the organism from the seeds have been unsuccessful. The common occurrence of lesions on the second leaf of seedlings points toward either seed or soil transmission.

In 1924 a few water-soaked partly red lesions, like those from Kansas, were found on some milo growing in the cereal plots at the Arlington Experiment Farm. Isolations from these lesions gave typical raised lemon-yellow colonies, but their pathogenicity was not proved. This is the only time the disease has been found at the Arlington farm, and it probably was introduced on the seed.

⁸ C. I. indicates a serial number of the Office of Cereal Crops and Diseases.

⁹ H. C. indicates a serial number used at Hays, Kans.

COMPARISON WITH OTHER BACTERIAL DISEASES OF SORGHUM

Lesions caused by *Bacterium andropogoni*¹⁰ on sorghum are never water-soaked and translucent, but even the youngest lesions are always red throughout, and no oval spots with tan centers and red borders develop. While lesions caused by *Bact. holcicola* have a reddish cast, there is much more brown in them than in those due to *Bact. andropogoni*. The exudate from lesions due to *Bact. andropogoni* forms red crusts, whereas that from lesions of *Bact. holcicola* forms thin white to cream-colored scales.

The disease caused by *Bacterium holci* Kendrick¹¹ on species of *Holcus* is characterized by light-centered, red-bordered, round to elliptical or irregular lesions on the leaves. They vary from 1 to 8 mm. in diameter. Very small lesions are red throughout. They resemble lesions of the disease here described in that at first they are dark green and water-soaked, but the long narrow red streaks of the present disease are neither described nor illustrated by Kendrick.¹²

Pseudomonas alboprecipitans Rosen¹³ on *Holcus* species produces grayish green spots bordered by red bands, or merely by small red irregular spots.

The organism described in this paper differs from the three mentioned above in important cultural, morphological, and physiological characters, which are summarized in Table 3.

TABLE 3.—Comparative cultural, morphological, and physiological characters of various bacterial organisms which produce lesions on *Holcus* spp.

Character compared	<i>Bacterium andropogoni</i>	<i>Bacterium holci</i>	<i>Pseudomonas alboprecipitans</i>	<i>Bacterium holcicola</i>
Color on agar.....	White.....	White fluorescent.	White.....	Yellow.
Size (μ).....	1.3-2.5 by 0.4-0.8..	1.5-2.9 by 0.6-1.0..	1.8 by 0.6.....	1.58 by 0.75
Oxygen relation.....	Aerobic.....	Aerobic.....	Aerobic.....	Aerobic.
Nitrates reduced.....	No.....	Yes.....	Yes.....	(?).
Ammonia produced.....	No.....	No.....	Yes.....	Yes.
Indol produced.....	No.....	No.....	No.....	No.
Hydrogen sulphide produced.	No.....	No.....	No.....	Yes.
Acid produced with dextrose.	Yes.....	Yes.....	No.....	No
Diastatic action.....	Yes.....	No.....	Yes.....	Yes.
Thermal death point (°C.)	48°.....	49°.....	41°-43°.....	51°.
Gram, negative or positive.	Negative.....	Negative.....	Negative.....	Negative.
Gelatin liquefied.....	No.....	Yes.....	No.....	Yes.
Milk cleared.....	Without curd.....	Without curd.....	Without curd.....	Without curd.

SUMMARY

Bacterial streak disease of sorghum is characterized by narrow reddish-brown streaks on the leaves. These lesions are water-soaked in early stages and in later stages may broaden at intervals into elongated oval spots with tan centers and narrow red margins. Exudate is abundant and dries to form thin white to cream-colored scales. Specimens have been collected in Texas, Oklahoma, Kansas, and Montana.

¹⁰ ELLIOTT, C., and SMITH, E. F. A BACTERIAL STRIPE DISEASE OF SORGHUM. Jour. Agr. Research 38: 1-22, illus. 1929.

¹¹ KENDRICK, J. B. HOLCUS BACTERIAL SPOT OF ZEA MAYS AND HOLCUS SPECIES. Iowa Agr. Expt. Sta. Research Bul. 100, p. 303-334, illus. 1926.

¹² KENDRICK, J. B. Op. cit.

¹³ ROSEN, H. R. A BACTERIAL DISEASE OF FOXTAIL (*CHAETOCLOA LUTESCENS*). Ann. Missouri Bot. Gard 9: 333-402, illus. 1922.

The disease spreads from younger to older leaves, but apparently it does not check the development of the plant sufficiently to make control measures necessary.

Experimental lesions were produced on Dwarf White milo by spraying with cultures of the organism isolated from young lesions on the same variety collected at Chillicothe, Tex. The organism isolated from lesions on Johnson grass and sprayed on Dwarf White milo after dark has also produced abundant typical infections, thus establishing its pathogenicity.

From the lesions has been isolated a yellow polar-flagellate organism which reproduces the disease when sprayed on healthy plants. This organism, which apparently is undescribed, is given the name *Bacterium holcicola* n. sp.

A study of this organism has been made; its morphology, staining reactions, cultural characters, and physiology are discussed, and a technical description is given.

Some tests of varietal susceptibility of sorghum to the infection were carried out. While the means of dissemination of the infection was not discovered, evidence is adduced to indicate that it spreads by seed or soil transmission. A tabular comparison was made between the cultural, morphological, and physiological characters of *Bacterium holcicola* and those of three other organisms which also attack sorghum, *Bact. holci*, *Bact. andropogoni*, and *Pseudomonas alboprecipitans*.

BEEF EXTRACT AS A SOURCE OF VITAMIN G¹

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INTRODUCTION

Beef extract is a highly concentrated water extract of fresh lean beef. It consists of those constituents of lean beef which are soluble in water and are not coagulated by heating. In other words, beef extract is concentrated beef broth. As regards nutritive value, beef extract is considered a food adjuvant rather than an important source of nutrients. Beef extract is rich in the flavoring constituents of meat and therefore has important culinary properties. Physiologically it has been found to exert a favorable effect on the flow of digestive fluids. Although 1 pound of solid beef extract contains the water-soluble constituents from 20 or more pounds of lean beef, beef extract has not been found to be a good source of the so-called water-soluble B vitamin. This is in harmony with the fact that lean beef is not well supplied with this vitamin complex.

The recent experiments by Goldberger and his associates² which showed that the so-called water-soluble B vitamin really consisted of at least two vitamins—one, the antineuritic, labile to heat, and the other stable to heat and without antineuritic properties—suggested to the writers that commercial beef extract might be a good source of the second vitamin. Goldberger designated the heat-stable vitamin as the P-P factor, meaning pellagra preventive, but in the interest of simplicity in nomenclature a committee of the American Society of Biological Chemists³ has recommended that the term "vitamin G" be used to denote the more heat-stable, water-soluble dietary factor, and that the term "vitamin B" be restricted to the antineuritic vitamin. The above terminology has been followed in this paper.

The purpose of the experiments reported in this paper was to determine the relative amounts of growth-promoting vitamin G in commercial beef extract obtained from different manufacturers.

METHODS

The relative amounts of vitamin G in samples of beef extract were estimated by feeding tests with young albino rats. Each ration containing a definite proportion of beef extract, calculated in terms of moisture-free extract, was fed to a group of four to six rats which were usually selected from three litters. Each rat was kept in an individual cage with raised screen bottom. The ration was supplied in a self-feeder and an accurate record was kept of feed consumed. Rats weighing approximately 40 grams each and not exceeding 28

¹ Received for publication Jan. 21, 1930, issued May, 1930.

² GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. M. A FURTHER STUDY OF BUTTER, FRESH BEEF, AND YEAST AS PELLAGRA PREVENTIVES, WITH CONSIDERATION OF THE RELATION OF FACTOR P-P OF PELLAGRA (AND BLACK TONGUE OF DOGS) TO VITAMIN B. [U. S.] Pub. Health Rpts. 41: 297-318, illus. 1926.

³ SEIDELL, A., SHERMAN, H. C., LEVENE, P. A., STEENBOCK, H., MCCOLLUM, E. V., and DUTCHER, R. A. REPORTS. VITAMIN B TERMINOLOGY. Science (n. s.) 69: 276. 1929.

days in age were first fed a basal ration practically free from vitamins B and G until growth ceased. The rats were then fed the test ration consisting of the basal ration supplemented with an alcoholic extract of white corn as the source of vitamin B (antineuritic vitamin) and beef extract as the source of vitamin G. The rats were weighed twice weekly.

Basal ration for rats

Casein (N×6.25).....	per cent..	20
Ash mixture.....	do.....	4
Cod-liver oil.....	do.....	2
Hydrogenated cottonseed oil.....	do.....	8
Cassava starch to make.....	do.....	100

Finely ground commercial casein was thoroughly extracted with 60 per cent alcohol by percolation and then dried. The ash mixture was made up according to a formula by Drummond.⁴ The cod-liver oil was a high-grade medicinal product. The hydrogenated cottonseed oil was a well-known commercial product. The cassava starch was a high-grade commercial product.

PREPARATION OF CORN EXTRACT

An alcoholic extract of corn as the source of vitamin B was prepared in a manner similar to that described by Goldberger and his associates.⁵ Five kilograms of finely ground white corn were mixed with approximately 12 liters of 85 per cent by volume of ethyl alcohol. The mixture was stirred at intervals, allowed to stand overnight, and then transferred to percolators. The filtrate was concentrated on a steam bath with the aid of a blast of air directed over the surface of the liquid. When most of the alcohol had evaporated and protein was precipitating from solution, the dish was removed from the steam bath and 650 grams of cassava starch was mixed with the concentrated extract. The mixture was spread out in a thin layer in shallow pans and dried at 60° C. or lower in an oven with a forced draft. The dried material was ground fine and stored in covered jars. Five kilograms of corn meal yielded approximately 730 grams of dried extract; hence 1 gram of extract corresponded to approximately 6.8 grams of corn.

DESCRIPTION OF BEEF EXTRACT

Pure concentrated beef extract was obtained from five meat-packing establishments operating under Federal inspection. For purposes of identification these establishments will be designated by the letters V, W, X, Y, and Z. Each lot of extract was first dissolved in water in order to facilitate mixing with the other constituents of the ration. The proportion of solids in the diluted extract was determined, and a sufficient quantity to furnish the desired proportion of extract in the ration was mixed with the calculated amount of starch. The mixture was dried and combined with the other constituents of the ration.

⁴ DRUMMOND, J. C., and COWARD, K. H. RESEARCHES ON THE FAT-SOLUBLE ACCESSORY SUBSTANCE. V. THE NUTRITIVE VALUE OF ANIMAL AND VEGETABLE OILS AND FATS CONSIDERED IN RELATION TO THEIR COLOUR. *Biochem. Jour.* 14: [668]-677. 1920.

⁵ GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. M. *Op. cit.*

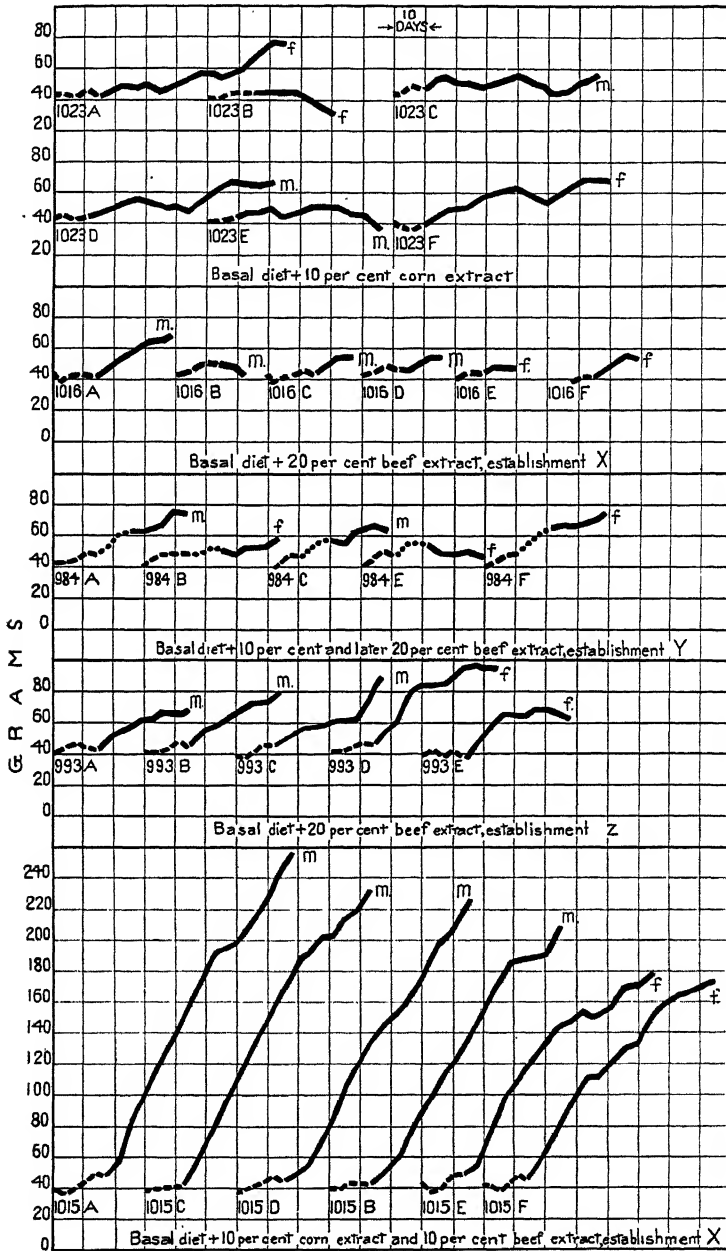


FIGURE 1.—Growth curves of rats showing effects of a deficiency of vitamins B and G, respectively, on growth. The broken lines indicate results of preliminary feeding on a diet very deficient in both vitamins B and G. The letters *m* and *f* denote sex. Rats Nos. 1023A to 1023F, inclusive, were fed a diet very deficient in vitamin G; rats Nos. 1016A to 1016F, 984A to 984F, and 993A to 993E, inclusive, were fed diets very deficient in vitamin B, while rats Nos. 1015A to 1015F, inclusive, were fed diets containing adequate amounts of both vitamins B and G.

RESULTS OF EXPERIMENTS

The basal ration supplemented with 10 per cent of corn extract as the source of vitamin B (antineuritic) was tested at intervals during these experiments to determine whether an appreciable amount of vitamin G was present. In all these tests the rats made but slight, if any, growth, indicating that the ration contained only a very small amount of this vitamin. On the other hand, when the ration was supplemented with autoclaved yeast as the source of vitamin G, the rats made excellent growth. These results show that the corn extract supplied an adequate amount of vitamin B for growth.

In Figure 1 the first group of curves (Nos. 1023A to 1023F, inclusive) shows the results obtained by feeding rats a ration adequate for normal growth except for lack of vitamin G. The rats were first fed the basal ration lacking both vitamins B and G until growth ceased (broken lines) when corn extract was added to the ration as a source of vitamin B (continuous lines). The failure of these rats to grow indicates that the ration did not contain an appreciable amount of vitamin G.

Three groups of rats—Nos. 1016, 984, and 993 (fig. 1)—were fed rations containing 20 per cent each of beef extract from establishments X, Y, and Z, respectively, as the sole addition to the basal ration. The slight growth made by these rats indicated that these samples of beef extract were very deficient in either vitamin B or vitamin G, or both.

Rats Nos. 1015A to 1015F, inclusive, the growth curves of which are shown at the bottom of Figure 1, were fed a ration containing 10 per cent of beef extract from establishment X as the source of vitamin G, the diet being adequate in other respects for normal growth. The excellent growth made by these rats indicates that 10 per cent of this lot of beef extract supplied an ample quantity of vitamin G. It is apparent, then, that the factor which limited the growth of rats Nos. 1016A to 1016F, inclusive, which received 20 per cent of the same lot of beef extract as the source of both vitamins B and G, was lack of vitamin B alone. Evidence to be presented later will show that lack of vitamin B was also the limiting factor in the growth of rats Nos. 984A to 984F, inclusive, and rats Nos. 993A to 993E, inclusive.

In Figures 2 to 6, inclusive, are shown the growth curves of rats which were fed rations containing beef extract from establishments V, W, X, Y, and Z, respectively, as the source of vitamin G. Beef extract from each establishment was fed at three levels of intake, viz, 5, 7.5, and 10 per cent, the percentage being calculated in terms of moisture-free extract. In Table 1 are presented data concerning the growth and feed consumption of these rats.

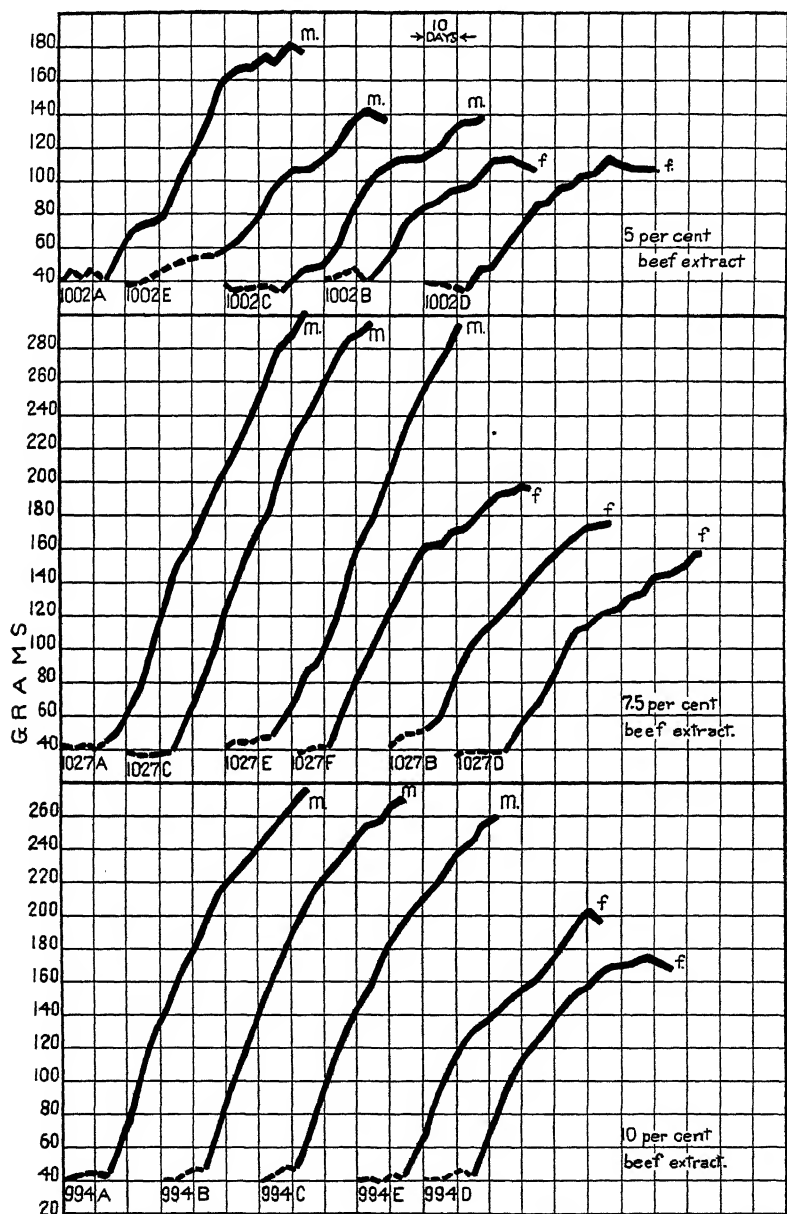


FIGURE 2.—Growth curves of rats fed diets containing beef extract from establishment V as the source of vitamin G

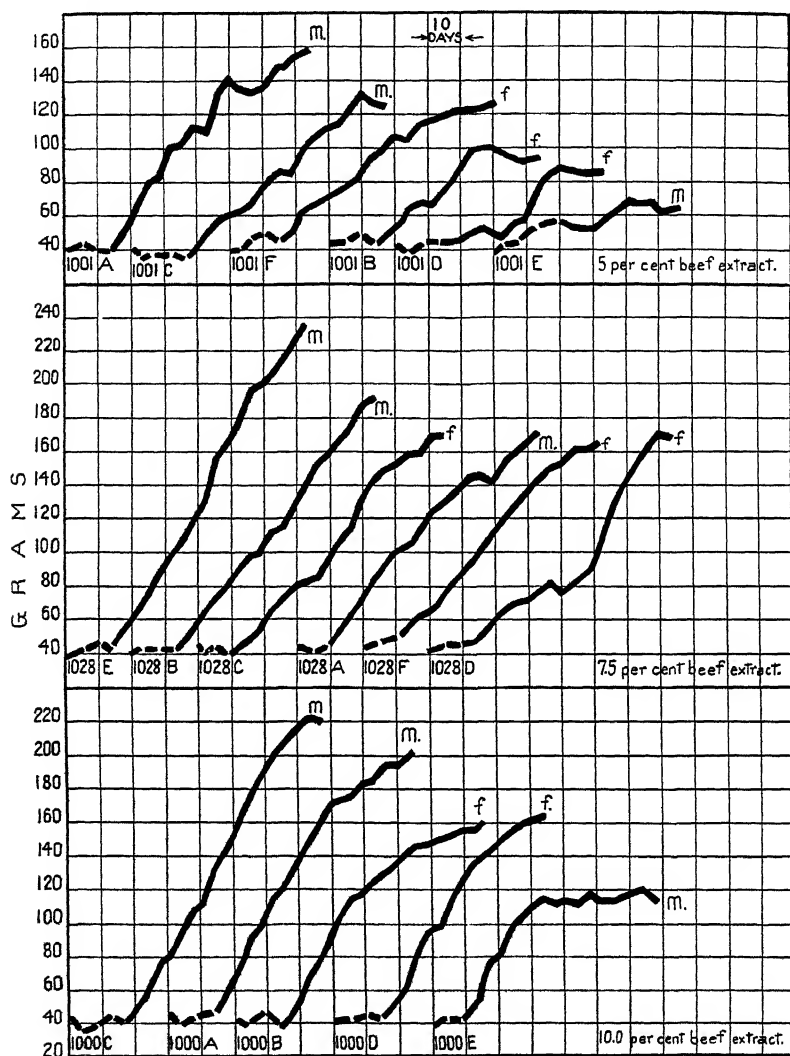


FIGURE 3.—Growth curves of rats fed diets containing beef extract from establishment W as the source of vitamin G

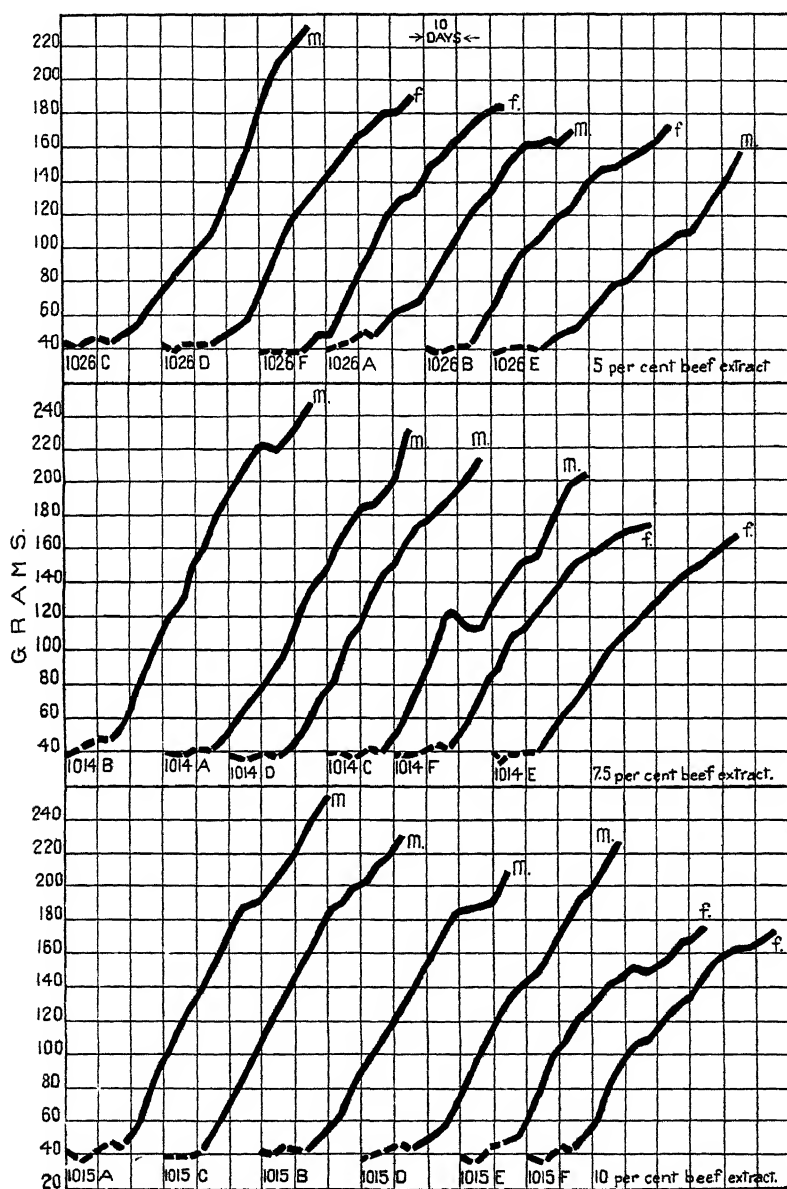


FIGURE 4.—Growth curves of rats fed diets containing beef extract from establishment X as the source of vitamin G

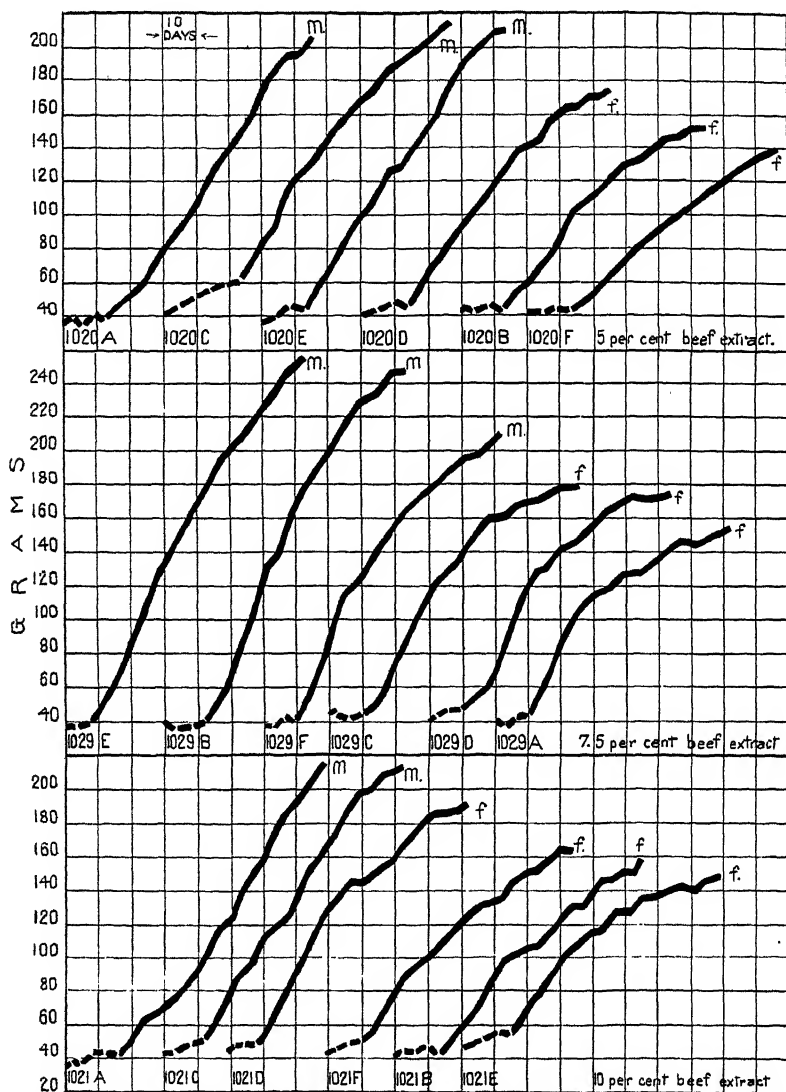


FIGURE 5.—Growth curves of rats fed diets containing beef extract from establishment Y as the source of vitamin G

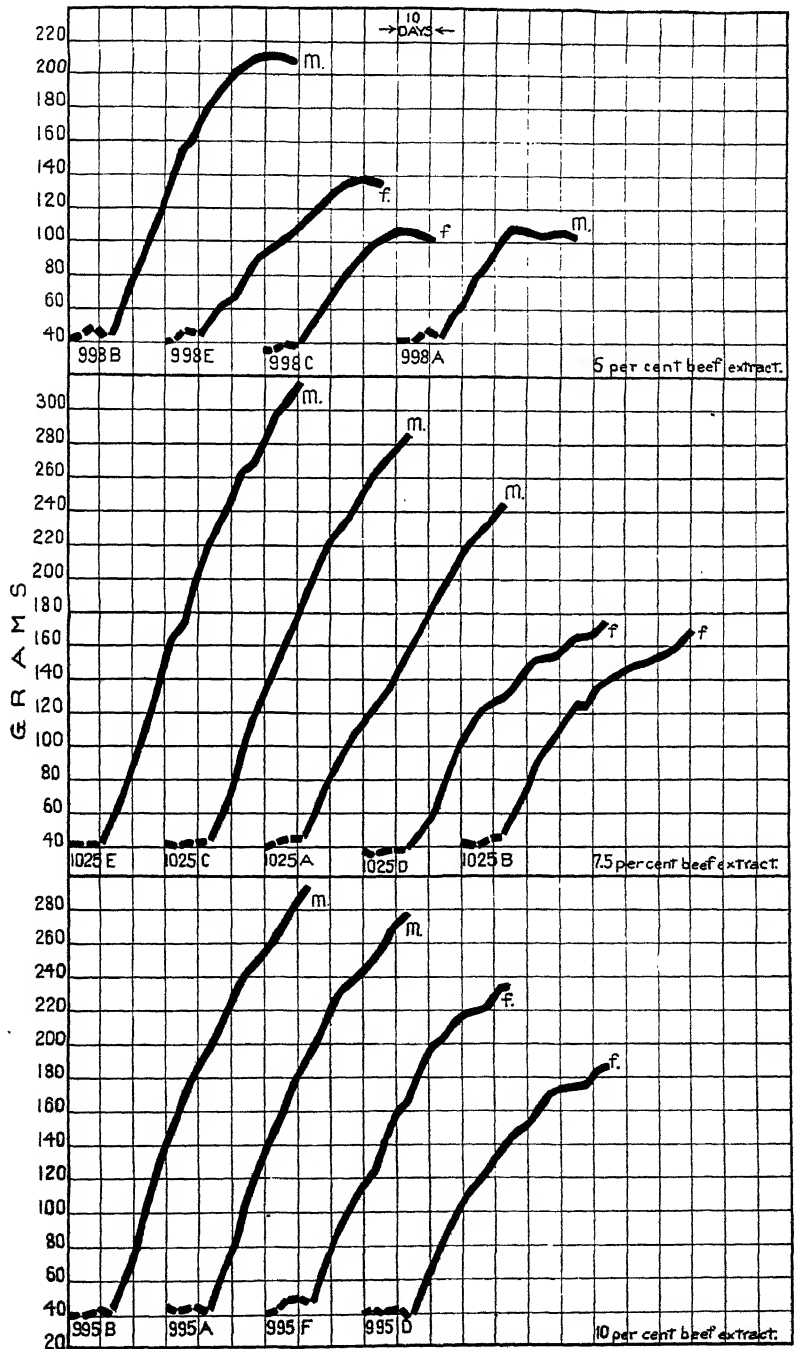


FIGURE 6.—Growth curves of rats fed diets containing beef extract from establishment Z as the source of vitamin G

TABLE 1.—*Feed consumed and gains made by rats fed different quantities of beef extract as a source of vitamin G*

Addition to basal ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total beef extract consumed	Average daily intake of beef extract	Average gain in weight per gram of feed eaten
			<i>Days</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Gram</i>
5 per cent beef extract (establishment V).	1002A	Male.....	60	137	2.28	517	25.85	0.43	0.265
	1002B	Female.....	49	69	1.41	319	15.95	.33	.216
	1002C	Male.....	60	104	1.73	384	19.20	.32	.271
	1002D	Female.....	56	73	1.30	332	16.60	.30	.220
	1002E	Male.....	52	82	1.58	375	18.75	.36	.219
7.5 per cent beef extract (establishment V).	1027A	Male.....	60	258	4.30	721	54.08	.90	.358
	1027B	Female.....	60	125	2.08	516	38.70	.65	.242
	1027C	Male.....	60	257	4.28	780	58.50	.98	.329
	1027D	Female.....	60	121	2.02	471	35.33	.59	.257
	1027E	Male.....	56	246	4.39	665	49.88	.89	.370
10 per cent beef extract (establishment V).	1027F	Female.....	60	158	2.63	607	45.53	.76	.260
	994A	Male.....	60	233	3.88	577	57.70	.96	.404
	994B	Female.....	60	224	3.73	645	64.50	1.08	.347
	994C	Male.....	60	214	3.57	633	63.30	1.06	.338
	994D	Female.....	60	126	2.10	529	52.90	.88	.238
5 per cent beef extract (establishment W).	994E	Male.....	60	158	2.60	540	54.00	.90	.289
	1001A	Male.....	60	119	1.98	517	25.85	.43	.230
	1001B	Female.....	49	51	1.04	294	14.70	.30	.173
	1001C	Male.....	60	92	1.53	422	21.10	.35	.218
	1001D	Female.....	49	43	.88	217	10.85	.22	.198
7.5 per cent beef extract (establishment W).	1001E	Male.....	38	8	.21	168	7.90	.21	.051
	1001F	Female.....	62	79	1.27	436	21.80	.35	.081
	1028A	Male.....	62	125	2.02	502	37.65	.61	.249
	1028B	Female.....	62	151	2.44	503	37.73	.61	.300
	1028C	Male.....	62	130	2.10	522	39.15	.63	.249
10 per cent beef extract (establishment W).	1028D	Female.....	62	123	1.98	435	32.63	.53	.283
	1028E	Male.....	60	199	3.32	594	44.55	.74	.335
	1028F	Female.....	60	116	1.93	481	36.08	.60	.241
	1000A	Male.....	60	156	2.60	583	58.30	.97	.268
	1000B	Female.....	60	123	2.05	530	53.00	.88	.232
5 per cent beef extract (establishment X).	1000C	Male.....	60	181	3.02	592	59.20	.99	.306
	1000D	Female.....	49	121	2.47	415	41.50	.85	.292
	1000E	Male.....	60	71	1.18	373	37.30	.62	.190
	1026A	Female.....	60	122	2.03	499	24.95	.42	.244
	1026B	Male.....	63	132	2.10	528	26.40	.42	.250
7.5 per cent beef extract (establishment X).	1026C	Female.....	60	188	3.13	575	28.75	.48	.327
	1026D	Male.....	60	149	2.48	561	28.05	.47	.266
	1026E	Female.....	60	117	1.95	377	18.85	.31	.310
	1026F	Male.....	60	147	2.45	550	27.50	.46	.267
	1014A	Female.....	60	190	3.17	519	38.93	.65	.366
10 per cent beef extract (establishment X).	1014B	Male.....	60	201	3.35	677	50.78	.85	.297
	1014C	Female.....	61	166	2.72	583	43.73	.72	.285
	1014D	Male.....	60	177	2.95	528	39.60	.66	.335
	1014E	Female.....	60	123	2.13	475	35.63	.59	.269
	1014F	Male.....	60	129	2.15	476	35.70	.60	.271
5 per cent beef extract (establishment Y).	1015A	Female.....	61	210	3.44	634	63.40	1.04	.331
	1015B	Male.....	60	169	2.82	448	44.80	.75	.377
	1015C	Female.....	60	190	3.17	600	60.00	1.00	.317
	1015D	Male.....	61	183	3.00	527	52.70	.86	.347
	1015E	Female.....	60	130	2.17	498	49.80	.83	.261
7.5 per cent beef extract (establishment Y).	1015F	Male.....	61	129	2.11	493	49.80	.82	.259
	1020A	Female.....	61	166	2.72	535	26.75	.44	.310
	1020B	Male.....	61	108	1.77	484	24.20	.40	.223
	1020C	Female.....	63	148	2.35	621	31.05	.49	.238
	1020D	Male.....	60	127	2.12	512	25.60	.43	.248
10 per cent beef extract (establishment Y).	1020E	Female.....	60	165	2.75	543	27.15	.45	.304
	1020F	Male.....	60	95	1.58	380	19.00	.32	.250
	1029A	Female.....	60	113	1.88	550	41.25	.69	.205
	1029B	Male.....	62	207	3.34	662	49.65	.80	.313
	1029C	Female.....	62	135	2.18	573	42.99	.69	.236
5 per cent beef extract (establishment Z).	1029D	Male.....	62	128	2.06	585	43.88	.71	.219
	1029E	Female.....	62	216	3.48	693	51.98	.84	.312
	1029F	Male.....	60	167	2.78	642	48.15	.80	.260
	1021A	Female.....	62	173	2.79	529	52.90	.85	.327
	1021B	Male.....	60	116	1.93	443	44.30	.74	.262
7.5 per cent beef extract (establishment Z).	1021C	Female.....	60	165	2.75	563	56.30	.94	.298
	1021D	Male.....	62	143	2.31	579	57.90	.93	.247
	1021E	Female.....	63	95	1.51	481	48.10	.76	.198
	1021F	Male.....	62	111	1.79	526	52.60	.85	.211
	998A	Female.....	42	59	1.40	242	12.10	.29	.244
5 per cent beef extract (establishment Z).	998B	Male.....	56	166	2.96	531	26.55	.47	.313
	998C	Female.....	42	63	1.50	230	11.50	.27	.274
	998E	Male.....	56	93	1.66	359	17.95	.32	.259

TABLE 1.—*Feed consumed and gains made by rats fed different quantities of beef extract as a source of vitamin G—Continued*

Addition to basal ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total beef extract consumed	Average daily intake of beef extract	Average gain in weight per gram of feed eaten
			<i>Days</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Gram</i>
7.5 per cent beef extract (establishment Z).	1025 A	Male.....	60	198	3.30	622	46.65	0.78	0.318
	1025 B	Female.....	60	126	2.10	550	41.25	.69	.229
	1025 C	Male.....	60	242	4.03	773	57.98	.97	.313
	1025 D	Female.....	60	136	2.27	504	37.80	.63	.273
	1025 E	Male.....	60	277	4.62	831	62.33	1.04	.333
10 per cent beef extract (establishment Z).	995 Ado.....	60	236	3.93	660	68.00	1.10	.358
	995 Bdo.....	60	251	4.18	673	67.30	1.12	.373
	995 D	Female.....	60	148	2.47	501	50.10	.84	.295
	995 Fdo.....	60	165	2.75	566	56.60	.94	.292

Referring to the growth curves at the top of each of Figures 2 to 6, inclusive, one may observe that 5 per cent of beef extract from establishments X and Y, respectively (figs. 4, 5), furnished sufficient vitamin G for good but not for normal growth. On the other hand, the same proportion of beef extract from establishments V, W, and Z, respectively (figs. 2, 3, 6), induced only poor to fair growth.

From Table 1 it appears that the three male rats which were fed 5 per cent of beef extract from establishment X (fig. 4) made an average daily gain of 2.37 grams and the three female rats a gain of 2.34 grams. The average daily consumption of beef extract by the male rats was 0.40 gram and by the female rats 0.45 gram.

Five per cent of beef extract from establishment Y induced an average daily gain of 2.60 grams in three male rats and 1.82 grams in three female rats. The average daily intake of beef extract by the male rats was 0.46 gram and by the female rats 0.38 gram.

Seven and one-half per cent of beef extract from establishments V, X, Y, and Z, respectively, promoted good to excellent growth in rats (figs. 2, 4, 5, and 6), while the same proportion of extract from establishment W (fig. 3) induced only fair growth. Some of the male rats made extraordinary growth. (Figs. 2, 6.)

From Table 1 the average daily gains made by the male rats fed rations containing 7.5 per cent of beef extract from establishments V, W, X, Y, and Z, respectively, were calculated to be as follows: 4.32, 2.59, 3.05, 3.20, and 3.98 grams. The average daily gains made by the female rats fed the same rations were as follows: 2.24, 2.00, 2.14, 2.04, and 2.19 grams, respectively. The average daily consumption of beef extract by the male rats which were fed rations containing 7.5 per cent beef extract from the above-named establishments was as follows: 0.92, 0.65, 0.72, 0.81, and 0.93 gram, respectively. The average daily consumption of beef extract by the female rats receiving the same rations was as follows: 0.67, 0.59, 0.60, 0.70, and 0.66 gram, respectively.

The data for the rats fed rations containing 10 per cent of beef extract need not be discussed in detail. An examination of Figures 2 to 6, inclusive, shows that the rats fed this proportion of beef extract did not, on an average, make more rapid growth than those receiving 7.5 per cent of beef extract from the same establishment.

Reference was previously made to the very poor growth made by three groups of rats fed rations containing 20 per cent each of beef extract from establishments X, Y, and Z, respectively, as the source of both vitamins B and G (fig. 1, rats 1016A to 1016F, 984A to 984F,

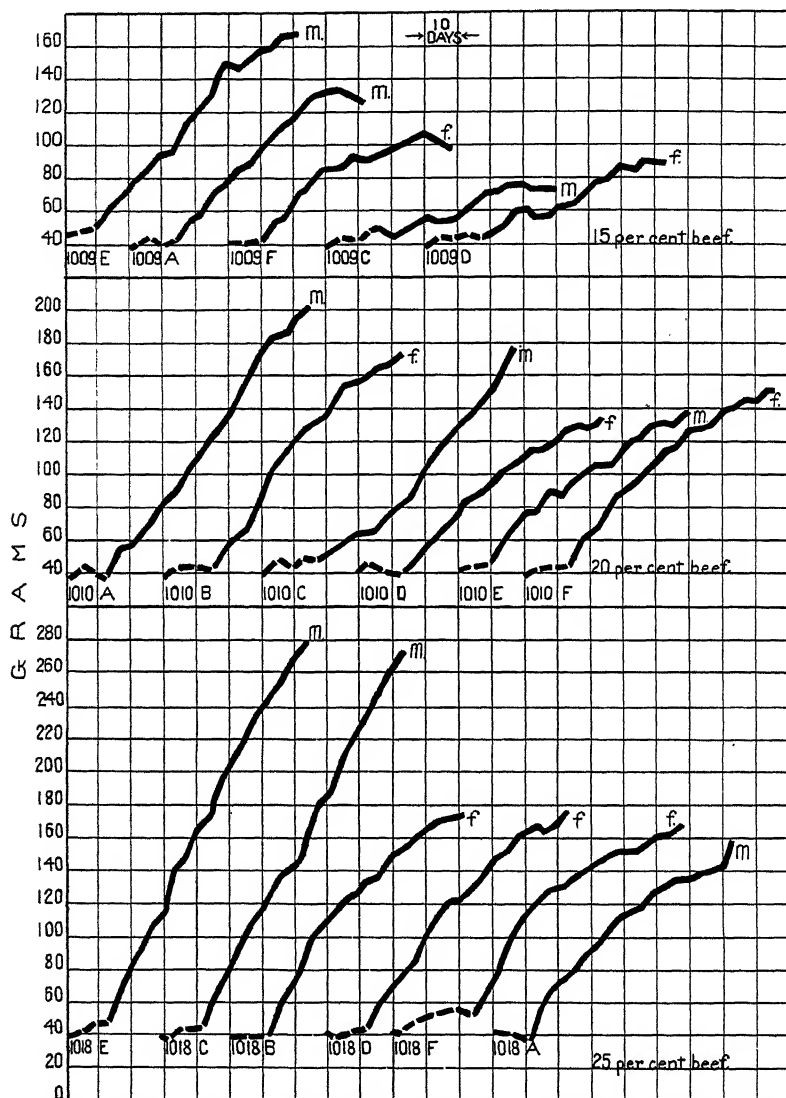


FIGURE 7.—Growth curves of rats fed diets containing dried lean beef as the source of vitamin G

and 993A to 993E). Evidence was presented which showed that the very poor growth made by the rats fed beef extract from establishment X was due to a deficiency of vitamin B. On referring to Figures 5 and 6, one will note that rats fed rations containing 7.5 per cent of beef extract from establishments Y and Z, respectively, as the source of

vitamin G, the ration containing an adequate amount of vitamin B, made normal growth. Hence, the very poor growth made by the rats getting 20 per cent of beef extract from these establishments as the source of both vitamins B and G was due to a deficiency of vitamin B.

VITAMIN G IN BEEF

For the purpose of comparing the amount of vitamin G in beef extract with that in lean beef, there are shown in Figure 7 the growth curves of rats fed different proportions of dried lean beef as a source of this vitamin. These graphs indicate that neither 15 nor 20 per cent of dried beef furnished sufficient vitamin G for normal growth. Twenty-five per cent of beef induced excellent growth in all but one rat. In Table 2 are presented data concerning the growth and feed intake of these rats.

TABLE 2.—Record of gains made by rats fed different quantities of lean beef as a source of vitamin G

Addition to basal ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total beef consumed	Average daily intake of beef	Average gain per gram of feed eaten
			<i>Days</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Gram</i>
15 per cent dried lean beef.	1009A	Male....	56	84	1.50	425	63.75	1.14	0.198
	1009Cdo....	57	23	.40	244	36.60	.64	.094
	1009D	Female...	56	47	.84	280	42.00	.75	.168
	1009E	Male....	60	117	1.95	546	81.90	1.37	.214
	1009F	Female...	56	54	.96	382	57.30	1.02	.141
	1010A	Male....	60	166	2.77	524	104.80	1.75	.317
20 per cent dried lean beef.	1010B	Female...	60	128	2.13	529	105.80	1.76	.242
	1010C	Male....	60	129	2.15	426	85.20	1.42	.303
	1010D	Female...	60	83	1.55	444	88.50	1.48	.209
	1010E	Male....	60	83	1.55	416	83.20	1.39	.224
	1010F	Female...	60	108	1.80	471	94.20	1.57	.229
	1018A	Male....	60	120	2.00	513	128.25	2.14	.234
25 per cent dried lean beef.	1018B	Female...	60	136	2.27	532	133.00	2.22	.256
	1018C	Male....	60	224	3.73	688	172.00	2.87	.326
	1018D	Female...	60	134	2.23	492	128.00	2.05	.272
	1018E	Male....	60	231	3.85	747	186.75	3.11	.309
	1018F	Female...	63	116	1.84	559	139.75	2.22	.208

The male rats receiving 25 per cent of dried beef made an average daily gain of 3.19 grams and the female rats an average gain of 2.11 grams. The average daily consumption of dried beef by the male rats was 2.71 grams and by the female rats 2.16 grams. For comparison, the average daily consumption of beef extract by the male rats receiving 7.5 per cent of extract in their diet was 0.80 gram and by the female rats 0.65 gram. These data indicate that the daily intake of dried beef necessary to furnish sufficient vitamin G for normal growth in male or female rats was approximately three and four-tenths times as large as the required amount of beef extract. These comparisons are made between moisture-free beef extract and air-dry beef. If the comparison is made between commercial beef extract containing approximately 20 per cent of moisture and fresh lean beef with a moisture content of 75 per cent, then 1 part of beef extract appeared to contain approximately as much vitamin G as 11 parts of lean beef.

DISCUSSION OF RESULTS

As regards the antipellagric potency of beef extract, the writers have no direct information, but the following comparison may throw some light on the subject. Goldberger and Tanner⁶ found that dried yeast in daily doses of 30 grams was very effective in preventing pellagra in human subjects for at least a year. In later experiments with yeast extract, Goldberger and his associates⁷ found that a daily dose of 15 grams of this product was as effective in preventing pellagra as 30 grams of yeast. The yeast extract was simply a dried, water-extract of yeast. Goldberger and his associates have also tested the pellagra-preventive action of fresh lean beef. The daily administration of 200 grams of fresh lean beef, which corresponds to approximately 50 grams of moisture-free beef, fully protected human subjects against pellagra for at least a year. Since yeast extract has been found to be much more effective in preventing pellagra than yeast, it may be that beef extract will likewise be more effective against pellagra than beef, comparisons being made between moisture-free products.

SUMMARY

Experiments were conducted with albino rats to determine the relative amounts of vitamin G in commercial beef extract obtained from five manufacturers. For comparison fresh lean beef was examined also.

It was found that an intake level of 7.5 per cent of moisture-free beef extract from each of four establishments furnished sufficient vitamin G for good to excellent growth in rats. The same percentage of beef extract from the other establishment promoted fair growth. In these experiments the average daily intake of moisture-free beef extract was approximately 0.80 gram for males and 0.65 gram for females.

Twenty per cent of dried lean beef furnished sufficient vitamin G for fair growth and 25 per cent enough for excellent growth in rats. The average daily intake of 25 per cent dried beef amounted to 2.7 grams for male and 2.2 grams for female rats.

One pound of moisture-free beef extract appeared to contain approximately the same amount of vitamin G as 3.4 pounds of dried beef. If the commercial products are compared, then 1 pound of concentrated beef extract contained approximately the same amount of vitamin G as 11 pounds of fresh, lean beef.

⁶ GOLDBERGER, J., and TANNER, W. F. A STUDY OF THE PELLAGRA-PREVENTIVE ACTION OF DRIED BEANS, CASEIN, DRIED MILK, AND BREWERS' YEAST WITH A CONSIDERATION OF THE ESSENTIAL PREVENTIVE FACTORS INVOLVED. [U. S.] Pub. Health Rpts. 40: 54-80. 1925.

⁷ GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. M. Op. cit.

CAROTENOSIS OF BOVINE LIVERS ASSOCIATED WITH PARENCHYMATOUS DEGENERATION¹

By JOHN S. BUCKLEY, *Chief, Pathological Division*; E. C. JOSS, *Assistant Chief, Meat Inspection Division*; G. T. CREECH, *Associate Veterinarian, Pathological Division*; and JAMES F. COUCH, *Chemist, Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

In the course of post-mortem inspection at slaughtering establishments operating under Federal meat inspection, various pathological conditions in bovine livers are observed by veterinary inspectors. Most of these conditions are of common occurrence, are recognized quite readily, and disposition in such cases can usually be made on the macroscopic appearance of the liver lesions. It occasionally happens, however, that a condition is observed which is immediately recognized as something out of the ordinary but can not be definitely diagnosed by gross examination of the affected liver.

During the last year the attention of the Meat Inspection Division was directed to an unusual condition in the livers of cattle, the outstanding gross characteristic of which was an intense yellow or reddish-yellow coloration of the liver tissue, while all other organs and tissues in the carcass, except the associated hepatic lymph gland which showed a yellowish mottled appearance, were normal in color and general appearance.

In cooperation with the Meat Inspection Division, the Pathological Division undertook an investigation of the liver condition in order to determine, if possible, the cause of the coloration. A request was sent to a number of inspectors in charge of slaughtering establishments for specimens of the yellow livers.

On receipt of the first lot of specimens, one of the writers (Creech) recalled that several livers similar in appearance had been received at the pathological laboratory a number of years previously. The laboratory records showed that the lot of yellow livers referred to had been sent to the laboratory in 1920 by J. S. Jenison, inspector in charge, National Stock Yards, Ill., for a determination of the cause of the yellow color.

The histological findings in the specimens from Jenison were "well-marked parenchymatous degeneration with round-cell infiltration and limited fibrous proliferation." Chemical studies of this lot of livers were made at the same time by W. N. Berg, formerly of the Bureau of Animal Industry. He found the pigmentation, or yellow coloration, to be due to "a substance identical with carotin." When these findings were made the condition was considered as being somewhat rare, and no further studies of yellow livers were made at that time.

Specimens of the yellow livers studied during the present investigations were received from five cattle-slaughtering centers, viz,

¹ Received for publication Jan. 15, 1930; issued May, 1930.

Chicago, Ill., Fort Worth, Tex., Kansas City, Kans., Buffalo, N. Y., and Phoenix, Ariz.

GROSS APPEARANCE OF AFFECTED LIVERS

The typical yellow livers have a characteristic appearance and should not be confused with livers showing other off-color conditions seen more frequently, such as bile discoloration in icteric conditions or yellowish, fatty livers. The affected livers are usually slightly enlarged, with well-rounded borders. In the early stages of the conditions the one outstanding characteristic, and in some cases practically the only deviation from the normal that can be detected in the gross specimen, is the intense yellow or reddish-yellow color of the liver tissue.

When fresh specimens of these livers are sectioned and handled, the knife and fingers are stained a deep yellow, which is distinctly different from the greenish-yellow tinge in bile-pigment staining.

As the pathological changes become more advanced the livers undergo fibrous changes. It has been observed that these fibrous proliferations may vary in the different liver specimens from a sprinkling of slight, fibrous areas barely visible in the gross specimen to advanced cirrhosis involving a large portion, possibly from one-third to one-half of the organ. The extreme cirrhotic changes are usually accompanied by more or less calcification.

In all cases examined in which the associated hepatic lymph glands accompanied the liver specimen, these glands exhibited a peculiar, yellowish, mottled appearance. The unusual deep-yellow color, with fibrous changes in some cases, as already indicated, and the lack of definite knowledge as to the cause of the condition, have led to the application of various terms, more or less descriptive, such as icteric liver, lutein liver, alkali liver, and Pictou disease, in designating this type of liver.

It appears that the condition is not confined to any particular age or sex. Some of the livers examined were from steers 2 to 3 years of age while others were from cows possibly 5 years old or older. The condition has been observed also in livers of aged range bulls.

It is of particular interest to note that no other abnormal changes are seen in any other organs or tissue of carcasses in which the yellow livers are found, with the exception, as previously indicated, of the hepatic gland. The fat and other tissue do not show any yellowish pigmentation, thus indicating that in all probability the condition is confined to the liver and associated lymph gland.

The history obtained thus far regarding the origin of cattle with yellow livers indicates that the condition is found most frequently, if not altogether, in cattle from the southwestern part of the United States.

CAUSE OF THE YELLOW PIGMENTATION

The close similarity between the livers examined in the present study and the specimens of yellow livers observed in 1920, in which the intense yellow color was found to be due to a substance identical with carotene, led to the belief that this was the same liver condition. It was decided, therefore, to make a chemical analysis of a number of specimens to identify definitely the coloring matter present.

CHEMICAL STUDIES

It was considered necessary to reinvestigate the coloring matter present in these livers because Berg had not published the evidence on which he based his conclusion that the substance was carotene; moreover, it was not certain that the more recent condition was the same as that studied by him. The coloring matter, therefore, was isolated in quantity and identified by chemical methods.

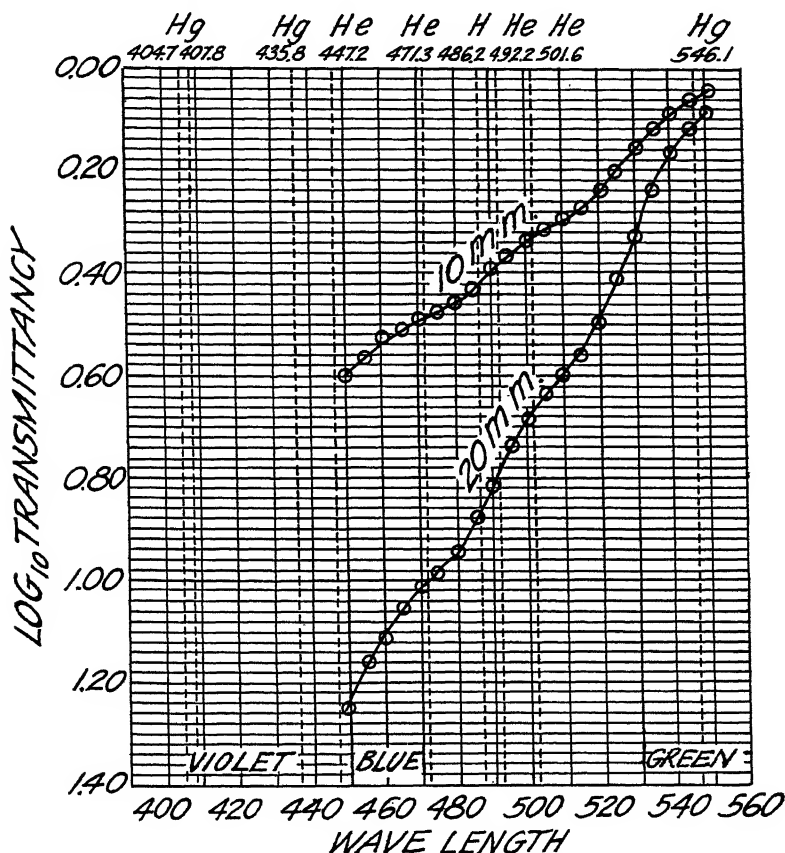


FIGURE 1.—Spectrophotometric measurements of a 0.05 per cent solution of carotene in carbon disulphide

A composite sample of livers 1, 2, and 3, amounting to 4,110 gm., was finely minced in a meat chopper, placed in a 12-liter flask, and 4 liters of 5 per cent alcoholic potash were added. The whole was boiled four hours under reflux, when nearly all the liver tissue was liquefied. The product was strained through cheesecloth and the alcohol removed by distillation. When cold the aqueous residue was extracted with successive portions of petroleum ether until that solvent was no longer deeply colored. The combined petroleum-ether solutions were washed with 85 per cent alcohol to remove xanthophyll, if present, and other possible impurities, and then the petroleum ether

was removed by distillation. The residue was a blood-red, thick, unctuous mass. This was purified by boiling with 5 per cent alcoholic potash, removing the alcohol, extracting with petroleum ether, washing with 85 per cent alcohol, and distilling off the petroleum ether. The residue was dissolved in carbon disulphide and the solution poured into 5 volumes of alcohol. The precipitated carotene was collected, washed with alcohol, and warmed on a water bath to remove volatile impurities.

The substance so obtained was identified as carotene as follows: It responded to the ferric chloride and sulphuric acid color tests; it showed the solubility relationships characteristic of carotene; the triiodide prepared according to the method of Arnaud² melted at 138°-139° without decomposition. Palmer³ gives the melting point 136°-137°, which is sufficiently close for identification. Spectrophotometric measurements of a 0.05 per cent solution of the substance, made at the Bureau of Standards, United States Department of Commerce, are shown in Figure 1, through the courtesy of K. S. Gibson, of that bureau.

A number of determinations of the quantity of carotene present in yellow livers submitted for study, and in normal livers obtained from a local packing plant, were made. In these determinations the excellent method of Connor⁴ was used. The results are given in Table 1.

TABLE 1.—*Carotene content and condition of affected and normal livers*

CATTLE LIVERS

Date	Animal	Carotene content	Condition of liver
		<i>Mgm. per 100 gm.</i>	
Sept. 24, 1928	Steer 1	30.4	Yellow.
Do.	Steer 2	45.1	Do.
Do.	Steer 3	29.6	Do.
Sept. 26	Steer	1.3	Normal.
Do.	do.	1.01	Do.
Do.	do.	.90	Do.
Nov. 5	Steer 4	49.8	Yellow.
Do.	Steer 5	21.08	Do.
Do.	Steer 6	7.2	(Irrhotic.
Do.	Steer 7	6.43	Yellow.
Dec. 22	Cow 1	18.9	Do.
Do.	Cow 2	43.4	Do.
Feb. 14, 1929	Steer 8	16.6	Do.
Apr. 19	Steer 9	4.0	Do.

LIVERS OF RATS AFTER FEEDING ON TYPICAL YELLOW LIVERS

Oct. 31, 1928	Rat 1	0.48	Pale, with necrotic areas.
Do.	Rat 2	.8	Do.
Do.	Rat 3	(*)	Do.
Do.	Rat (control)	(*)	Normal.

* Trace.

² ARNAUD, A. RECHERCHES SUR LA COMPOSITION DE LA CAROTINE, SA FONCTION CHIMIQUE ET SA FORMULE. *Compt. Rend. Acad. Sc. [Paris]* 102: 1119-1122. 1886.

³ PALMER, L. S. CAROTINOIDS AND RELATED PIGMENTS; THE CHROMOLIPOIDES. p. 234. New York. 1922.

⁴ CONNOR, C. L. STUDIES ON LIPOCHROMES. III. THE QUANTITATIVE ESTIMATION OF CAROTIN IN BLOOD AND TISSUES. *Jour. Biol. Chem.* 77: 619-626. 1923.

In all cases search was made for other lipochromes, particularly xanthophyll, but none were found. Since carotene itself is not a toxic substance,⁵ further study is needed to determine what deleterious compound is present in these livers. The investigation is being continued in the Bureau of Animal Industry.

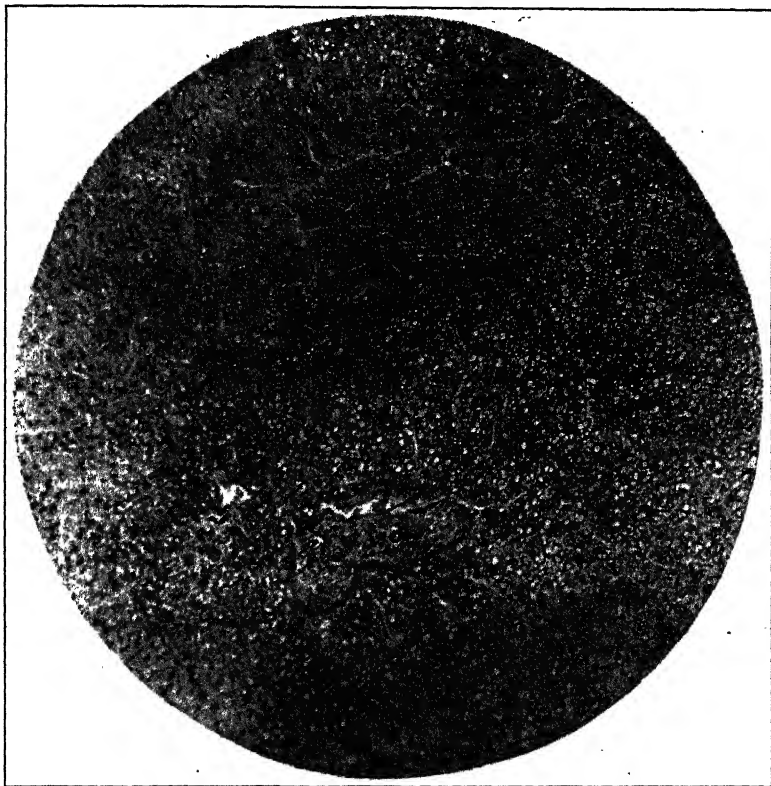


FIGURE 2.—Photomicrograph of section of liver of cow No. 2, showing degeneration of the liver cells. Many of the cells contain fat droplets. $\times 120$

HISTOPATHOLOGY

In the earlier stages of carotenosis of the liver the outstanding pathological change is that of a parenchymatous degeneration. The degenerative changes may vary in different parts of the same liver from a slight cloudiness, or granular appearance of the cell protoplasm, to complete degeneration and disintegration of groups of liver cells. In the well-advanced cases these areas of degeneration and necrosis are more extensive, including whole lobules or groups of lobules.

⁵ WELLS, H. G., and HEDENBURG, O. F. THE TOXICITY OF CAROTIN. *Jour. Biol. Chem.* 27: 213-216. 1916.

The degenerative changes appear to have their beginning most frequently in the central portion of the lobules. There is a very noticeable vacuolation of the liver cells in the degenerated areas, and frequently the cells, particularly toward the periphery of the affected lobules, contain numerous fat droplets. (Figs. 2 and 3.)

In a few of the cases examined practically no normal liver cells could be found in any of the sections made from various parts of the



FIGURE 3.—Photomicrograph of section of liver of steer No. 1, showing extensive degeneration of the cells. $\times 620$

specimens. (Fig. 4.) Some specimens show an engorgement of the central veins and capillaries with small hemorrhages here and there.

Areas of round-cell infiltration are frequently seen. These are the first indication of the fibrous changes which occur in the later stages of the disease. The fibrous changes are first seen in those areas showing advanced degeneration and in the region of the portal canals. The capsule of the liver may be very much thickened. In those cases showing advanced fibrosis, extensive proliferations of bile ducts were noted. (Fig. 5.) In two cases examined there was complete cirrhosis of considerable portions of the liver. (Fig. 6.) Large cal-

cified centers were noted in those areas showing the more advanced cirrhotic changes. Few of the livers examined histologically showed an excessive quantity of bile pigment.

The associated hepatic lymph glands exhibited very unusual and rather extensive degeneration of the gland cells. Few of the normal germinal centers could be recognized. A very large proportion of the gland cells had a peculiar, swollen appearance, and in a few places there were fusions of these cells and collections of nuclei simulating

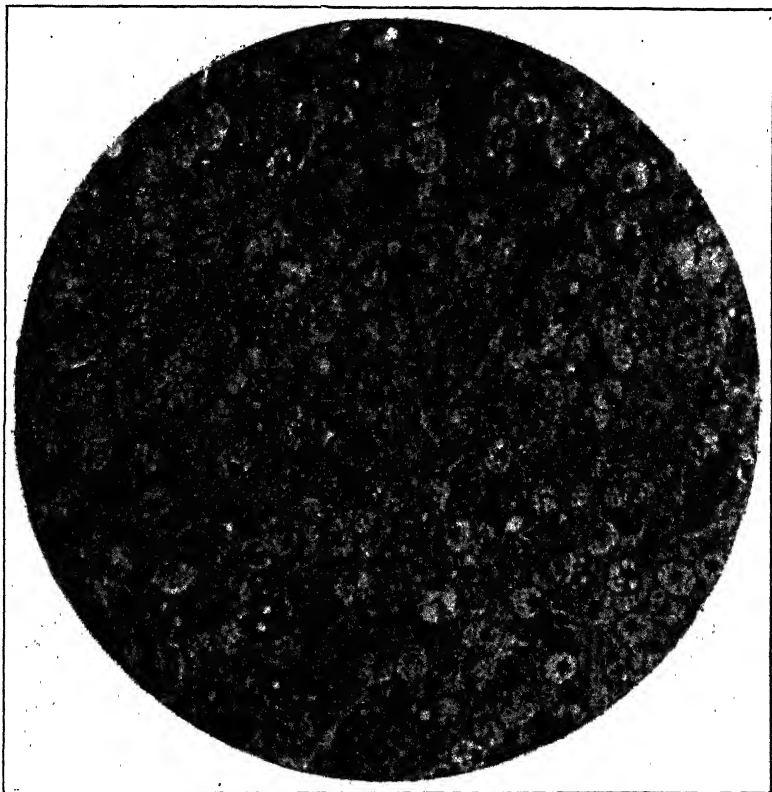


FIGURE 4.—Photomicrograph of section of liver of steer No. 4, showing advanced degeneration of the cells. Practically no normal cells were seen in this specimen. $\times 620$

giant-cell formation. Toward the central portion of the affected glands large areas of cells had been transformed into necrotic, homogeneous masses. (Fig. 7.)

The yellow coloring matter in the livers and lymph glands had evidently been largely removed by the alcohols in the preparation of the tissues for sectioning. Slight traces of the carotene deposits were noted in some of the degenerated and fatty cells of the hepatic lymph glands, but none of the coloring matter could be seen in the liver sections.

RAT-FEEDING EXPERIMENTS

Being unable to understand this rather peculiar proclivity of the bovine liver to store up an excessive amount of carotene while other tissues in the body remained unaffected, the writers decided to conduct some experimental rat feeding with typical yellow livers in order to determine whether these animals would show the same tendency to carotenosis of the liver.

In addition to the liver ration all of the experimental rats received daily a small quantity of corn meal and crushed oats mixed.

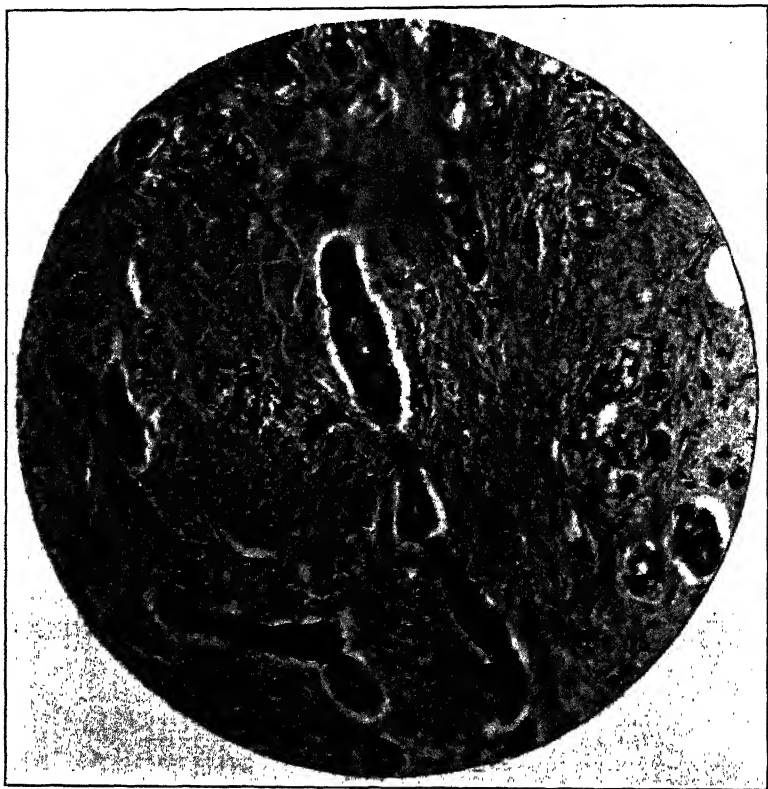


FIGURE 5.—Photomicrograph of section of liver of steer No. 6, showing advanced fibrous changes with extensive bile-duct proliferation. $\times 620$

As a preliminary experiment only three rats were fed. These were mature white rats and received 10 gm. daily of typical yellow liver from steer No. 2 during a period of approximately five weeks.

On post-mortem examination the livers of all the rats in this group were found to be very pale or light in color, but there was no distinct yellowish coloration of the liver tissue. Each liver, however, showed a number of small, whitish areas, evidently necrotic foci. On chemical analysis the three livers failed to show an excessive quantity of carotene, as may be observed in Table 1.

Histologically the livers showed an extensive degeneration of the liver cells. These degenerative changes were more in evidence immediately surrounding many of the central veins and larger blood vessels. (Fig. 8.) In places the complete degeneration and disintegration of the cells had resulted in small, necrotic centers which were seen in sections from all three of the livers. Groups of round cells were observed within and around some of these necrotic centers. (Fig. 9.)

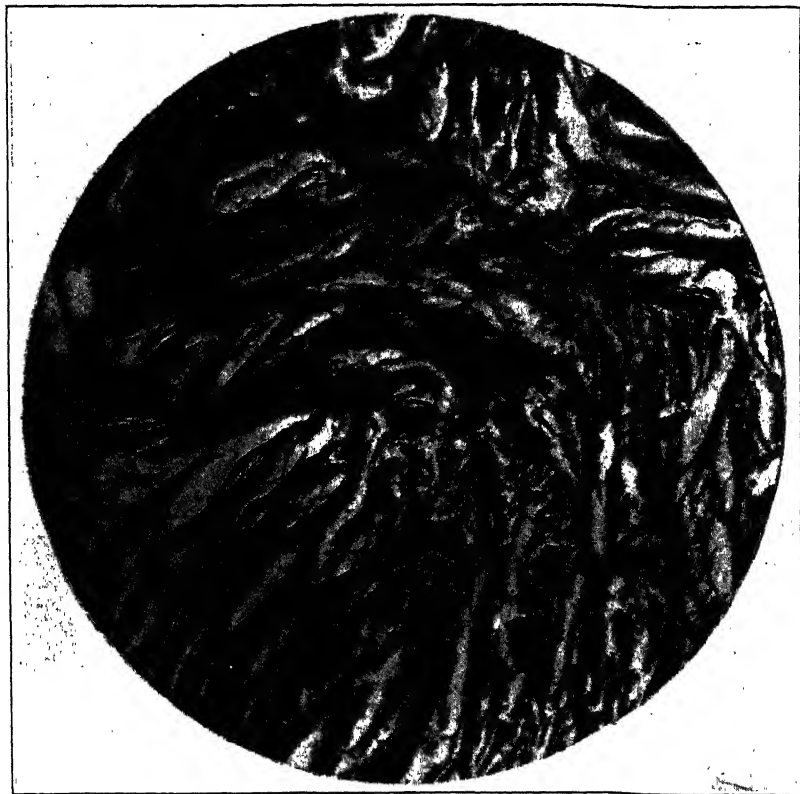


FIGURE 6.—Photomicrograph of section of liver of steer No. 5, showing the complete fibrosis which involved a large portion of the specimen. $\times 620$

While the preliminary feeding experiment gave negative results for carotene deposits, the very interesting findings with respect to the pathological changes in the livers of this small group of rats made it desirable to repeat the experiment in order to ascertain whether this same condition could be produced in a second or third lot of rats. Accordingly arrangements were made to feed two more lots of rats with yellow livers, and at the same time to feed a group of rats a similar quantity of normal bovine liver daily as controls in the experiment.

The rats used in the second feeding experiment were obtained from the Biochemic Division of the Bureau of Animal Industry, and more was known of their previous history and rationing than had been known in the case of the small group used in the preliminary experiment.

Ten rats were used in the second experiment. They were divided into two groups of three animals each, which were fed the yellow livers, and a third group of four rats, which were fed normal bovine

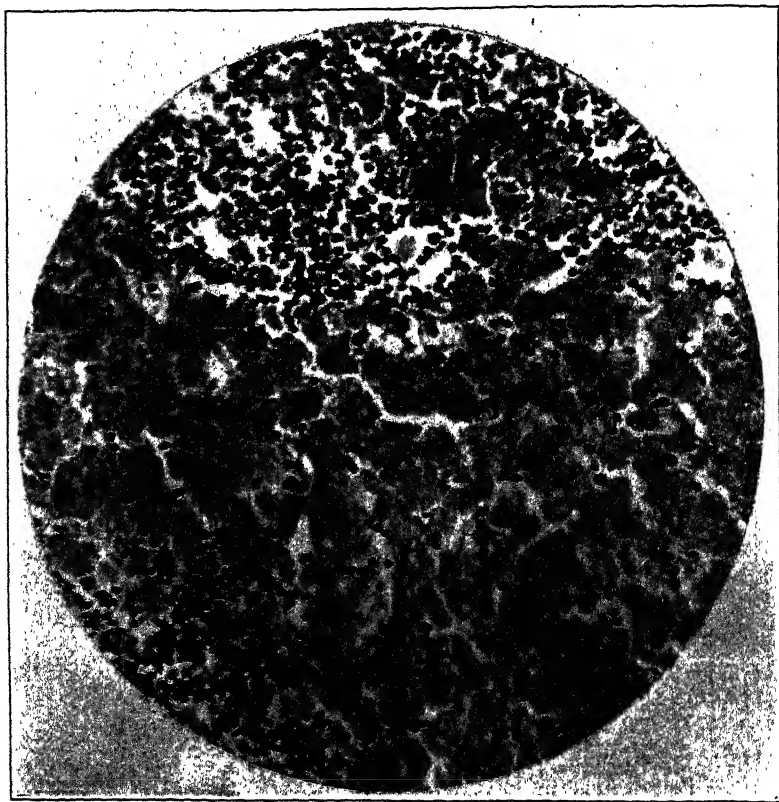


FIGURE 7.—Photomicrograph of section of hepatic lymph gland of steer No. 5, showing extensive areas of degeneration of the cells. $\times 620$

liver. These rats were quite young at the beginning of the experiment, averaging in weight about 40 gm. each.

Lots 1 and 2 were fed liver specimens from steers Nos. 4 and 5, respectively, which were typical cases of carotenosis. Since the rats were rather small they were fed only 5 gm. daily of the liver at first, but during the last half of the feeding period the quantity was increased to 10 gm. daily. Like quantities of normal bovine liver were fed to the four control rats for the same period.

The rats in lots 1 and 2, fed the carotene livers, averaged only 141 gm. in weight at the end of the feeding experiment, an increase of

101 gm., while the rats fed normal liver averaged nearly 154 gm. each, an increase of almost 114 gm.

The post-mortem findings in the livers of rats in lots 1 and 2 were substantially the same as those in the livers of the three rats used in the preliminary feeding experiment; that is, they consisted chiefly of extreme paleness and evidence of necrosis as indicated by numerous small, whitish areas scattered through the livers. The lesions were

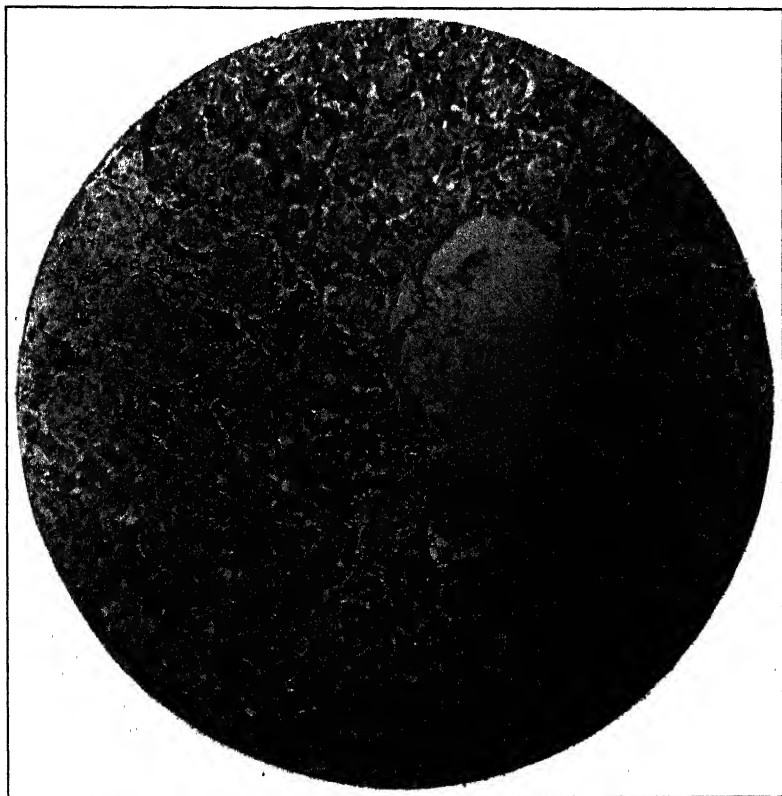


FIGURE 8.—Photomicrograph of section of liver of rat No. 3 (first experiment), showing numerous groups of degenerated cells. $\times 120$

somewhat more pronounced in the two lots of the second feeding than in the group of three animals in the first feeding.

The livers of all the rats in lots 1 and 2 were similarly affected, the only difference being the degree of involvement in the different animals. All other tissues of the rats in these two groups appeared to be normal.

The livers of all four of the rats in lot 3, fed normal bovine liver, were found on post-mortem examination to be normal in appearance, and the histological examination of these livers also showed them to be practically normal in structure and appearance. (Fig. 10.)

Histologically the livers of the rats in the two groups fed on carotene livers 4 and 5, in the second feeding experiment, showed more advanced degeneration and necrosis than the livers of the three rats in the preliminary feeding experiment. (Figs. 11 and 12.) The degenerative changes were found to be more extensive and the necrotic foci more numerous. In fact, in some of the specimens there was very little remaining normal liver structure.

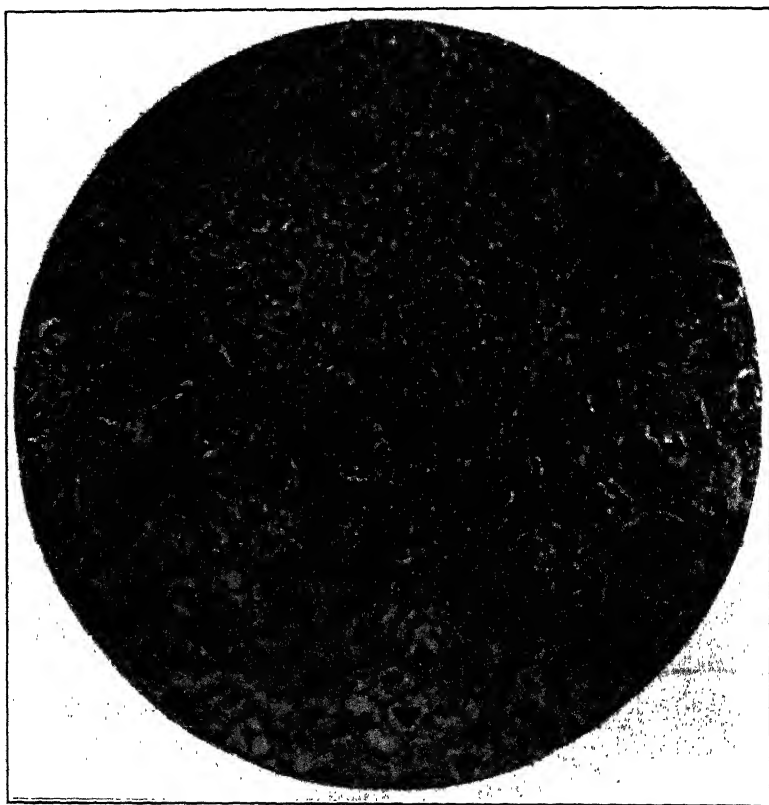


FIGURE 9.—Photomicrograph of section of liver of rat No. 1 (first experiment), showing degeneration of the cells and necrotic center with round-cell infiltration. $\times 620$

There was no decided yellowish coloration of any of the livers in either of the two groups of rats fed on yellow livers in the second experiment, and a chemical analysis of these livers did not show the presence of excessive quantities of carotene in any of the livers of the experimental rats.

ETIOLOGY

The pathological changes found in the different specimens of bovine liver showing carotenosis indicate that the destructive processes in these cases are the result of some form of acute irritation. However, the studies made thus far, which include bacteriological, histological,

and chemical examinations, have failed to reveal the nature of the causative agent.

That the liver changes are due to some specific, toxic substance and that this substance is stored up in the bovine liver tissue in appreciable quantities is shown by the fact that similar destructive changes can be induced in the livers of rats which have been fed for a time on affected cattle livers. The similarity of the degenerative changes in the various cases examined also indicates that the causative element is in all probability of a specific nature.

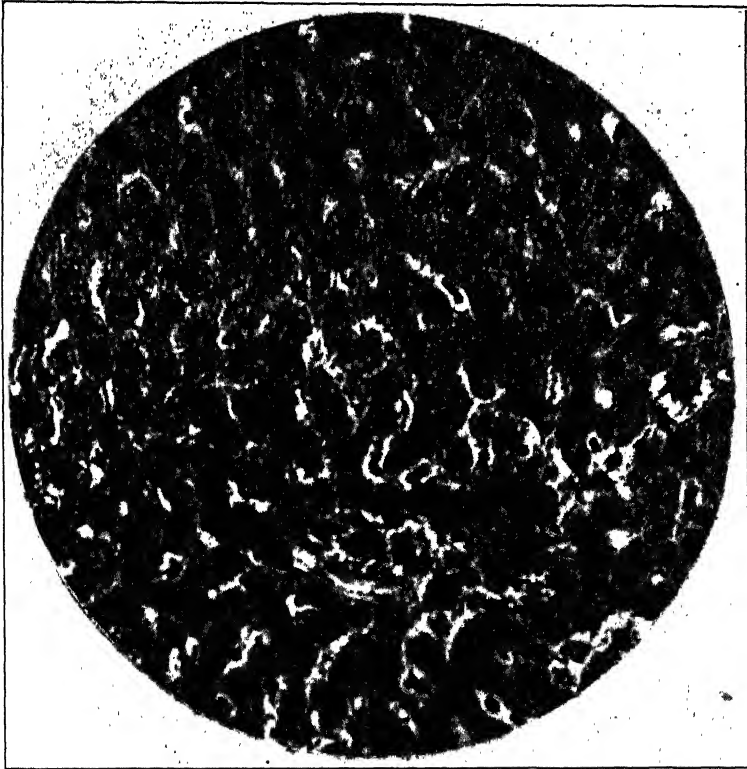


FIGURE 10.—Photomicrograph of section of liver of rat No. 7, control (second experiment). The liver of this rat and those of the other three controls were found to be practically normal. $\times 620$

The fact that the lesions are confined to the liver in cattle and also in the experimental rats suggests that the causative toxic substance has a peculiar affinity for the liver cells; at least it would appear from these observations that for some unknown reason the toxic substance does not pass beyond the liver after reaching that organ.

SUMMARY AND CONCLUSIONS

The results thus far obtained in studies of bovine livers showing carotenosis indicate that the destructive changes found in these livers are, in all probability, due to the presence of a toxic substance, the

nature of which has not yet been determined. That this substance is of a specific nature is indicated by the similarity of the lesions in the different cases studied.

In view of the evidence at hand and the limited available history of the cattle affected, the writers are of the opinion that the causative

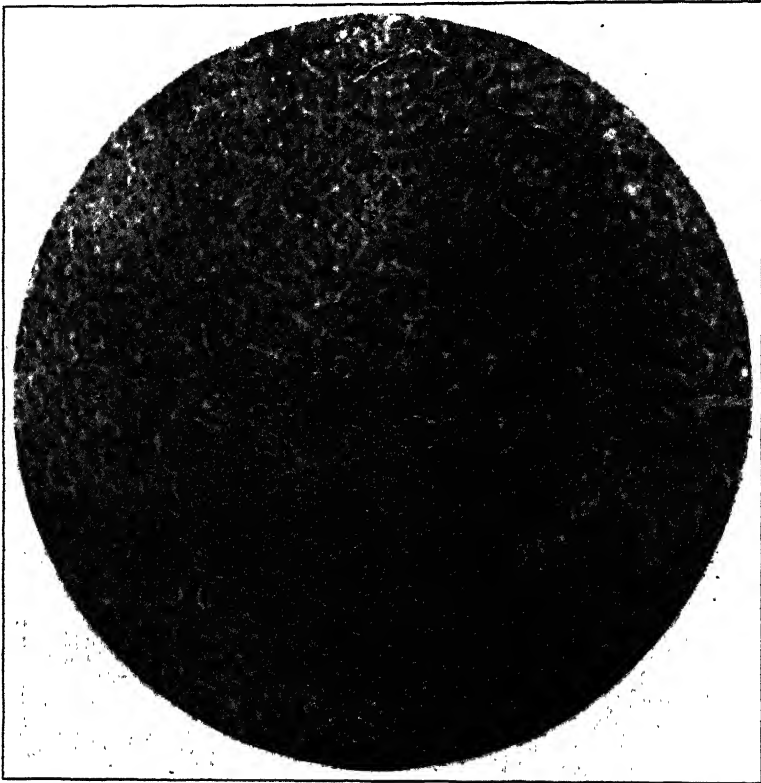


FIGURE 11.—Photomicrograph of section of liver of rat No. 3 (second experiment), showing areas of degenerated cells and one large center of necrosis. $\times 120$

agent, or toxic substance, is a constituent of some plant indigenous to the region or locality where the affected cattle had their origin.

The yellow coloring matter, or pigment, found in the affected livers has been definitely identified as carotene, and it is thought that the carotenosis in these cases is simply an associated condition in which, for some reason difficult to explain, the excess carotene is stored up in the liver, while other tissues of the body remain unaffected.

For the purpose of securing further information relative to this peculiar tendency of the bovine liver to store up carotene in large quantities, feeding experiments were carried out with rats. The rats were fed 5 or 10 gm. daily of typical yellow liver for some weeks. On post-mortem examination the livers of these rats were found to be very pale or light in color, though there was no distinct evidence

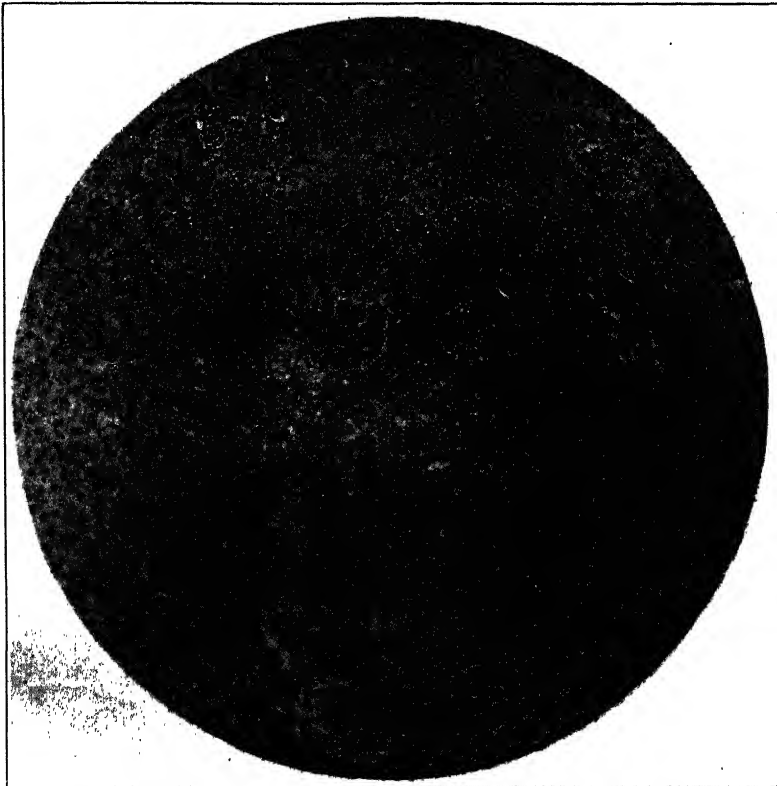


FIGURE 12.—Photomicrograph of section of liver of rat No. 1 (second experiment), showing areas of necrosis with round-cell infiltration. $\times 120$

of yellowing; each of the livers also showed a number of small, whitish areas, which were subsequently found to be necrotic foci. On chemical analysis, however, none of the livers of the experimental rats showed an abnormal quantity of carotene. Control animals fed on normal bovine liver grew more rapidly than animals fed yellow livers of high carotene content.

THE INSECTICIDAL ACTION OF SOME DERIVATIVES OF PYRIDINE AND PYRROLIDINE AND OF SOME ALIPHATIC AMINES¹

By C. H. RICHARDSON, formerly *Entomologist*,² and H. H. SHEPARD, *Assistant Entomologist, Division of Deciduous Fruit Insects, Bureau of Entomology, United States Department of Agriculture*³

NATURE OF THE STUDY

The investigation reported in this paper is a study of the relative toxicity of various nitrogenous organic compounds when used as contact insecticides. These compounds were synthesized by F. B. La Forge, of the Bureau of Chemistry and Soils, and submitted by him to the Bureau of Entomology for a study of their insecticidal value.⁴ Many of the compounds are related more or less closely to nicotine or to those simpler substances, pyridine and methylpyrrolidine, which are contained in the nicotine molecule. A few compounds are of insecticidal interest mainly for other structural characteristics of the molecule or as substances intermediate in the synthesis of some of the compounds.

This investigation has two objects: (1) To find some compound having a toxicity comparable to that of nicotine which can be synthesized for practical use; and (2) to make some contribution toward the explanation of the unique toxicity of nicotine. A considerable number of compounds have been investigated, very few of which show much toxicity, and none have the extreme toxicity of nicotine. Some of the more toxic compounds will be tested further.

EXPERIMENTAL METHODS

Essentially the same method has been used in the determination of toxicity as was employed by Richardson and Smith (10). Nasturtium plants bearing colonies of *Aphis rumicis* L. were set in bottles of water, the stems passing through perforated rubber stoppers. The sprays used were applied at a constant pressure of 5 pounds per square inch by an atomizer connected to an air-pressure line into which a pressure regulator and gauge were inserted. After the applications were made each plant was set aside on a paper bordered with a sticky tree-banding material to prevent the escape of the aphids. After any excess spray had drained down the stem a collar of cardboard was placed around each plant just above the stopper to prevent any of the aphids from falling into the accumulation of spray material at the base of the stem. After from 20 to 24 hours the percentage of aphids killed and the condition of the plants were determined. The figures in this paper are based on counts in which all sizes of aphids are included. Separate record has been made of the adult aphids killed in

¹ Received for publication Nov. 12, 1929; issued May, 1930.

² Resigned June 20, 1928.

³ The writers are indebted to R. C. Burdette for assistance in some of the experiments.

⁴ This is part of a cooperative project between the Insecticide Division, Bureau of Chemistry and Soils, and the Division of Deciduous Fruit Insects, Bureau of Entomology, the chemical part of which has already been published (4, 5, 6).⁵

⁵ Reference is made by number (italic) to Literature Cited, p. 1014.

the more recent tests. In the period from May, 1927, through June, 1928, over 65,000 aphids were counted, of which 11.7 per cent were adults.

Most of the compounds tested are miscible in water. A few had to be dissolved in a little acetone or other solvent and then shaken up in the spreader to emulsify them.

COMPARISON OF SOAP AND SAPONIN AS SPREADERS IN THE EXPERIMENTS

Richardson and Smith (10, p. 8) in 1923 reported that a spray of 0.3 per cent sodium fish-oil soap killed an average of 14 per cent of a population of *Aphis rumicis*. Since the date of these earlier experiments further tests of soap as a toxic agent have been made which tend to show that the earlier figure of toxicity was considerably too low and to suggest the use of some less toxic spreader for the tests. These later tests covered a period of about a year and showed a mean kill of 46.9 per cent in a total population of nearly 6,500 aphids used as controls. Still more recently a test of 0.3 per cent soap was made on a colony of about 400 aphids in which 44 per cent of all sizes and 28.8 per cent of the adults were killed.

Tattersfield, Gimingham, and Morris (14, p. 64; 15) used a 1 per cent saponin solution as a wetting agent in their tests of insecticidal action. In view of a considerable reduction in the toxicity due to the spreader when 1 per cent saponin is used, this material has been utilized in the later tests in the present investigation. Controls, in which over 2,500 aphids have been employed at various dates since 1925, showed when sprayed with 1 per cent saponin an average kill of 14.4 per cent of the aphids. There are other points relative to wetting and emulsification in which either soap or saponin excel. The relative toxicity of the wetting agent is, however, the character of the most importance here, and the other qualities will not be dealt with at this time.

BASIS OF COMPARISON OF EFFECTIVENESS OF SPRAYS

As in previous tests (10, p. 2) the toxic concentration has been "considered to be the minimum capable of killing about 95 per cent of the aphids." As the concentration which kills 50 per cent of the aphid population is a more valuable point, statistically, for comparative purposes, both this "median dose" to kill 50 per cent and the "practical dose" to kill 95 per cent have been tabulated.

The criterion of tolerance of the plant to a compound was the same as that described in the paper just cited (10, p. 2).

RESULTS OF THE EXPERIMENTS

The data here presented have been tabulated from curves which were made from all the observations, and the figures given in Tables 1 and 2 show the approximate (or actual) concentrations at which the mortality amounted to 50 or 95 per cent. Table 1 shows the results thus obtained when the spray carried 0.3 per cent of sodium fish-oil soap as a spreader and Table 2 the results obtained later with 1 per cent of saponin. The same compounds were not always available when the two series of tests were being made.

TABLE 1.—Toxicity of various compounds based on the concentration necessary to kill 50 and 95 per cent of *Aphis rumicis* on nasturtium leaves, and the plant tolerance shown, with 0.3 per cent of sodium fish-oil soap used as a spreader

Compound	Experiments	Range of concentrations tested	Toxic concentration necessary to kill approximately—		Tolerance of plant
			50 per cent	95 per cent	
	Number	Grams per 100 c. c.	Grams per 100 c. c.	Grams per 100 c. c.	Grams per 100 c. c.
Nicotine and closely related compounds:					
Nicotine.....	10	0.00025–0.05	^a (0.0003)	(0.007)	>0.05
Nicotyrine.....	4	.005 – .1	(.004)	.05	> .1
Metanicotine.....	5	.005 – .44	(.003)	.05	> .44
Dihydrometanicotine.....	5	.01 – .5	(.03)	.5	> .5
Methylmetanicotine.....	3	.01 – .1	< .01	.1	> .1
Benzoylmetanicotine.....	4	.01 – .2	.01	> .2	> .2
Pyridine and derivatives:					
Pyridine.....				25	25 –30
Pyridine vinyl bromide.....	3	.5 –2.0	2.0	>2.0	>2.0
Pyridine allyl bromide.....	7	.05 –5.0	(.03)	.25	.25 –.5
Dipyridine ethylene ammonium chloride.....	3	.25 –1.0	(.15)	1.0	1.0
β -pyridylcyanide.....	5	.25 –3.0	.5	>3.0	>3.0
β -pyridylethylamine.....	5	.05 –1.5	.3	1.5	>1.5
β -pyridylethyl-N-ethylamine.....	4	.01 –.5	.03	>.5	>.5
β -pyridyl-n-butylamine.....	4	.05 –1.0	<.1	>1.0	>1.0
β -pyridyl-n-butyl-N-methylamine.....	2	.5 –1.0	<.5	>1.0	>.5
Pyrrole, pyrrolidine and derivatives:					
Pyrrole.....	7	2.5 –18.0	5.0	18.0	2.5
Pyrroline hydrochloride.....	3	.1 –1.0	.1	(.8)	>1.0
Pyrrolidine hydrochloride.....	6	.1 –1.2	.1	1.0	.1
N-methylpyrrolidine hydrochloride.....	3	.1 –1.0	(.25)	1.0	1.0
N-ethylpyrrolidine hydrochloride.....	5	.1 –1.0	.25	1.0	.8
N-n-butylpyrrolidine hydrochloride.....	4	.1 –1.0	(.2)	.5	.5
N-benzylpyrrolidine.....	4	.1 –1.0	(.08)	(.4)	.25 –.5
α -methylpyrrolidine hydrochloride.....	5	.065 –1.0	(.12)	.5	.5
α -methyl-N-benzylpyrrolidine.....	4	.05 –.5	.1	.5	>.5
α -phenyl-N-methylpyrrolidine.....	5	.05 –2.0	(.8)	2.0	.25 –.5
α -phenyl-N-methylpyrrolidine.....	6	.1 –1.0	(.15)	(.6)	.5(?)
α -phenylpyrrolidine.....	3	.25 –1.0	<25	.5	(?)
α -dimethylpyrrolidine oxalate (neutral).....	3	.1 –1.0	(.08)	.5	.1
Aliphatic amines and derivatives:					
Vinyltrimethylammonium hydroxide (neutrine).....	3	.25 –1.0	(.2)	.5	0.25
Allyltrimethylammonium hydroxide.....	5	.05 –2.0	(.3)	1.0	<.5
Allyltrimethylammonium bromide.....	3	.5 –2.0	<.5	(.8)	.5
Phenyl-n-butyl-N-methylamine.....	4	.25 –2.0	.5	(1.5)	.25
Phenylallyl-N-methylmethylamine ^b	4	.25 –2.0	.5	(1.5)	.25 –.5

^a Figures in parentheses are estimated from points on the toxicity curves. The other figures are from readings very close to or directly at the points where the mortality was 50 or 95 per cent.

^b The compound designated by this name has 3 possible formulas (4); i. e., $C_6H_5CH_2(NHCH_3)CH_2CH=CH_2$, $C_6H_5CH=CHCH_2CH_2NHCH_3$, or $C_6H_5CH=CHCH(NHCH_3)CH_3$. The first one is phenylallyl-N-methylmethylamine.

TABLE 2.—*Toxicity of various compounds based on the concentration necessary to kill 50 and 95 per cent of Aphis rumicis on nasturtium leaves, and the plant tolerance shown, with 1 per cent of saponin used as a spreader*

Compound	Experiments	Range of concentrations tested	Toxic concentration necessary to kill approximately		Tolerance of plant
			50 per cent	95 per cent	
Nicotine and closely related compounds:	Number	Grams per 100 c. c.	Grams per 100 c. c.	Grams per 100 c. c.	Grams per 100 c. c.
Nicotine.....	8	0.002-0.2	(0.015)	(0.08)	>0.2
Metanictotine.....	3	.02-.5	(.04)	(.4)	>.5
Methylmetanictotine.....	3	.02-.5	(.02)	(.3)	>.5
Propylmetanictotine.....	3	.02-.5	.1	(.4)	.1
Pyridine derivatives:					
Pyridine vinyl bromide.....	2	.5-1.0	(1.5-2.0)	-----	>1.0
Pyridine allyl bromide.....	3	.02-.5	.1	>.5	>.5
β -2-allylpyridine.....	3	.02-.5	>.5	-----	>.5
β -Pyridylphenylmethylaniline.....	3	.02-.5	>.5	-----	>.5
Dimethyl- β -pyridyl carbinol.....	3	.02-.5	>.5	-----	>.5
Benzylpyridine, (b. p. 260°-280° C.).....	6	.005-.5	(.04)	.5	.2
Benzylpyridine (b. p. 280°-300° C.).....	4	.02-.5	.02	(.4)	.2
Benzylpyridine (b. p. 300° C. and over).....	3	.02-.5	(.05)	.5	.1
α -benzylpyridine.....	4	.005-.5	.02	.5	1-.5
β -benzylpyridine.....	3	.02-.5	.5	>.5	.1
γ -benzylpyridine.....	3	.02-.5	.1	>.5	>.5
Benzylpiperidine (b. p. 260°-270° C.).....	3	.02-.5	(.2)	>.5	>.5
α -dihydrostilbazol.....	3	.02-.5	.02	.5	.1
Pyrrolidine derivatives:					
Pyrrolidine hydrochloride.....	3	.1-1.0	(.7)	>1.0	(?)
α -methylpyrrolidine hydrochloride.....	3	.125-.5	(.4)	>.5	>.5

The most toxic compounds that have been tested in this study are nicotine and the closely related compounds, nicotyrine and metanictotine, which were synthesized from it. Nicotine is the only one of these three compounds that occurs in any great quantity in nature. The figures in Tables 1 and 2 show nicotyrine and metanictotine to be very close to each other in relative toxicity, nicotine being from 3 to 13 times as toxic, according to the spreader used in the test spray and whether the relative toxicity is based on the concentration needed to kill 95 or 50 per cent of the aphids. As a measure of this closeness one may compare the magnitude of other differences in toxicity; i. e., nicotine is about 100 times as toxic as dihydrometanictotine, 150 times as toxic as N-methylpyrrolidine hydrochloride and 3,500 times as toxic as pyridine. A few of the most toxic compounds are being examined by more exact methods than those employed in the tests described in this paper.

There are very few references of a quantitative nature to the pharmacological action of metanictotine and nicotyrine. Fränkel (2, p. 426), quoting Falck and Ringhardt (11), says metanictotine and nicotine act in the same manner, but a dose of metanictotine nine times a given dose of nicotine required twice as long in which to kill.

The toxicity figures for pyridine are taken for purposes of comparison from a previous paper (10, p. 4) where a discussion of the literature on the toxicity of that compound may be found. Pyrrole apparently has a toxicity of the same order as pyridine, whereas all the pyrrole and pyrrolidine compounds investigated are very much more toxic. The pyridine compounds studied, with the exception of the benzylpyridines and dihydrostilbazol, which will be discussed later, are in general less toxic than the pyrrole and pyrrolidine compounds.

TOXICITY AND CHEMICAL STRUCTURE

Richardson and Smith (10, *p. 11*) discussed the effect of hydrogenation of the cyclic nucleus of some compounds, to increase toxicity in the case of pyridine and piperidine, and to decrease it in the case of nicotine and hexahydronicotine. In the present investigation it has been found that the partial hydrogenation of pyrrole to pyrrolidine increases toxicity about as much as complete hydrogenation (or reduction) to pyrrolidine. Hydrogenation of α -phenyl-N-methylpyrrolidine to the corresponding pyrrolidine, however, makes it from 4 to 8 times as toxic. The increase in toxicity of pyrrolidine over pyrrole is considerably greater than in the change of pyridine to hexahydropyridine (piperidine). Nicotyrine (β -pyridyl- α -N-methylpyrrole) is from 7 to 10 times less toxic than nicotine (β -pyridyl- α -N-methylpyrrolidine). This increase in toxicity on the addition of hydrogen to unsaturated N-heterocyclic compounds is attributed by Fränkel (2, *p. 26*) to an increase in reactivity of the nitrogen when it passes from the tertiary to the secondary grouping in the reduction of pyridine. Oswald (7, *p. 326*) follows the preceding writer in saying that hydrogenation increases the activity of the nitrogen in pyridine. He adds, however, the statement that, in the case of pyrrole in which the nitrogen is secondary instead of tertiary as in pyridine, no considerable increase is discernible in its reduction to pyrrolidine, a point which is not upheld by the present results.

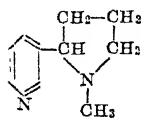
Oswald (7, *p. 328*) further reports that unsaturated side chains increase toxicity more than do saturated ones. This is difficult to harmonize with the decrease in toxicity which seems to be rather general in the case of unsaturated rings. However, the fact that the mere addition of two hydrogen atoms to satisfy a double bond in the side chain of metanicotine reduces the toxicity 100 times is in agreement with Oswald's statement.

Fränkel (2, *p. 66*) states that methylation of the nitrogen reduces toxic action. Oswald (7, *p. 328*), on the other hand, says that N-methylaniline and N-methylpiperidine are more active, respectively, than aniline and piperidine. Browning et al. (1, *p. 337*) report that substitution of methyl and ethyl radicles into NH_2 groups of diaminoacridine lessens the toxicity. Several instances of methylation may be cited among the compounds tested in the present investigation, but none show any effect upon toxicity that may be considered significant. Methyl-, ethyl-, and normal butyl-pyrrolidine hydrochlorides have about the same toxicity as the simple pyrrolidine hydrochloride. (Table 1.) β -pyridyl-n-butyl-N-methylamine has practically the same toxicity as β -pyridyl-n-butylamine. (Table 1.) The same is true in the case of α -phenyl-N-methylpyrrolidine and α -phenylpyrrolidine (Table 1) and in the case of metanicotine, methylmetanicotine, and propylmetanicotine (Table 2).

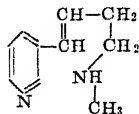
Methylation of the alpha carbon atom in N-benzylpyrrolidine produces a small but definite reduction in toxicity.

The cyclic pyrrolidine ring of nicotine may be replaced by an aliphatic amine group, as in metanicotine, with the destruction of only a part of the original toxicity of the former compound. It is therefore possible that other aliphatic amines of simpler structure might be substituted in the β position of pyridine to form compounds that would be highly toxic to insects.

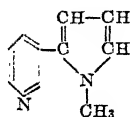
A few of the compounds tested, the structural formulas of which are similar to that for nicotine, are represented in the descending order of their toxicity as follows:



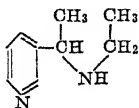
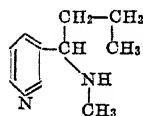
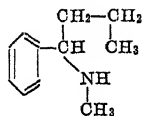
(1) Nicotine



(2) Metanictotine



(3) Nicotyrine

(4) β -Pyridylethylethylamine(5) β -Pyridyl-n-butyl-N-methylamine(6) β -Phenyl-n-butyl-N-methylamine

Oswald (8, p. 430, 436), referring to the pharmacological action qualitatively, says that α - α -pyridylpyrrole acts on frogs similarly to β -pyridyl-N-pyrrole and to β -pyridyl- α -N-methylpyrrole (nicotyrine). He also reports that nicotine (β -pyridyl- α -N-methylpyrrole) is much more toxic than nicotine.

Tattersfield (12) thinks "the whole make-up of the nicotine molecule, including its spatial arrangement, contributes to its toxic properties." Tattersfield and Gimmingham (13, p. 235) state that "nicotine undoubtedly owes its high potency to its molecular make-up taken as a whole; the attachment of the pyrrolidine nucleus in the β -position of the pyridine ring, and the presence of an asymmetric carbon atom may be significant."

TOXICITY OF BENZYLPIRIDINE

Preliminary tests of benzylpyridine showed that it held some promise as an effective insecticide that could be readily synthesized (La Forge (6), also Tattersfield and Gimmingham (13, p. 236)). As these tests were continued it became apparent that a large share of the toxicity might be due simply to the immiscibility in water (oily nature) of benzylpyridine, hence the comparative tests reported in Table 3 were made.

TABLE 3.—Comparison of effects of benzylpyridine (b. p. 270°–310° C.) and of a naphthenic-base petroleum oil (viscosity 105 seconds at 100° F.) sprayed on *Aphis rumicis* on nasturtium leaves

Spray composition	0.5 per cent spray		0.1 per cent spray		0.02 per cent spray	
	Total aphids in test	Mortality	Total aphids in test	Mortality	Total aphids in test	Mortality
Naphthenic base petroleum oil:	Number	Per cent	Number	Per cent	Number	Per cent
Emulsified with 1 per cent saponin.....	314	56.4	628	43.2	(?)	* 15
Emulsified with fish-oil soap (cold-mix formula)....	824	91.3	1, 027	51.1	-----	-----
Benzylpyridine:						
Emulsified with 1 per cent saponin.....	* 300	98+	334	74.8	-----	-----
Emulsified with fish-oil soap (cold-mix formula)....	733	98.5	1, 306	71.1	1, 261	31.7
Acid solution (1 per cent saponin as spreader).....	328	78.6	403	52.6	-----	-----
Acid solution emulsified with ammonia (1 per cent saponin as spreader).....	* 300	97+	245	75.9	-----	-----
Acid solution (0.3 per cent fish-oil soap as spreader)...	* 300	99+	369	89.2	-----	-----

* Estimated.

All the preliminary tests were made with spray solutions in which the required quantity of benzylpyridine was emulsified by shaking with a 10 per cent saponin solution and was then diluted to the required strength. These solutions had to be shaken frequently and used within an hour or two so that the oil would not separate out at the bottom of the flask. In all cases the 0.5 per cent solutions were milky emulsions, whereas the 0.1 and 0.02 per cent solutions were fairly clear.

It was found that the 1 per cent saponin emulsions were not suitable for properly comparing a petroleum oil with benzylpyridine. The naphthenic oil separated out in much larger oil globules than did the benzylpyridine at the same concentration. Hence a cold-mix soap emulsion of each material was made according to the formula of Richardson and Griffin (9), except that sodium fish-oil soap instead of potassium fish-oil soap was used. Table 3 shows that benzylpyridine has about the same effect in either type of emulsion notwithstanding the much greater toxicity of the soap. In the case of the naphthenic oil the soap emulsion still gives a toxicity somewhat below that of benzylpyridine but much higher than that of the imperfect saponin emulsion. The droplets of oil in the latter emulsion were larger and toxicity should have been higher (3), but because the oil droplets were so variable in size and so unevenly distributed in the solution, it was impossible to select samples which had the same oil concentration.

At the suggestion of Doctor La Forge benzylpyridine was dissolved in dilute hydrochloric acid and again thrown out of solution by neutralizing with dilute ammonia water, the benzylpyridine appearing in the form of extremely small droplets that remain suspended for several days.

Spray tests were made with these solutions and emulsions. It was found that a solution of benzylpyridine titrated with hydrochloric acid in the presence of methyl red in which there could be no complication owing to the presence of large oily droplets of benzylpyridine showed a greatly reduced toxic action, whereas the toxicity imme-

diately appeared again upon the addition of ammonia water, thus releasing the benzylpyridine in the form of tiny oil droplets.

SUMMARY

Numerous nitrogenous organic compounds have been tested as contact insecticides against *Aphis rumicis* L. colonized on nasturtium plants. These compounds for the most part are structurally related to nicotine.

Metanicotine and nicotyrine are the only substances tested which show a toxicity approaching that of nicotine.

The relation of chemical structure to toxicity, particularly hydrogenation and methylation of certain groups, is briefly discussed.

Benzylpyridine appears to have some promise as an insecticide for special uses where its oily properties are advantageous, and yet where an ordinary cheap oil is not suitable.

LITERATURE CITED

- (1) BROWNING, C. H., COHEN, J. B., GAUNT, R., and GULBRANSEN, R.
1922. RELATIONSHIPS BETWEEN ANTISEPTIC ACTION AND CHEMICAL CONSTITUTION WITH SPECIAL REFERENCE TO COMPOUNDS OF THE PYRIDINE, QUINOLINE, ACRIDINE AND PHENAZINE SERIES. Roy. Soc. London Proc. (B) 93: 329-366.
- (2) FRÄNKEL, S.
1906. DIE ARZNEIMITTEL-SYNTHESE AUF GRUNDLAGE DER BEZIEHUNGEN ZWISCHEN CHEMISCHEM AUFBAU UND WIRKUNG FÜR ÄRZT UND CHEMIKER. Aufl. 2, umgearb., 761 p. Berlin.
- (3) GRIFFIN, E. L., RICHARDSON, C. H., and BURDETTE, R. C.
1927. RELATION OF SIZE OF OIL DROPS TO TOXICITY OF PETROLEUM-OIL EMULSIONS TO APHIDS. Jour. Agr. Research 34: 727-738, illus.
- (4) LA FORGE, F. B.
1928. THE PREPARATION OF SOME PYRROLIDINE DERIVATIVES. Jour. Amer. Chem. Soc. 50: 2471-2477.
- (5) ———
1928. THE PREPARATION AND PROPERTIES OF SOME NEW DERIVATIVES OF PYRIDINE. Jour. Amer. Chem. Soc. 50: 2477-2483.
- (6) ———
1928. THE PREPARATION OF α -, β - and γ -BENZYL PYRIDINES. Jour. Amer. Chem. Soc. 50: 2484-2487.
- (7) OSWALD, A.
1921. DIE BEZIEHUNGEN ZWISCHEN CHEMISCHER KONSTITUTION UND PHYSIOLOGISCHER WIRKUNG. Schweiz. Chem. Ztg. 1921: [299]-303, [325]-329, [337]-341.
- (8) ———
1924. CHEMISCHER KONSTITUTION UND PHARMAKOLOGISCHE WIRKUNG, IHRE BEZIEHUNGEN ZU EINANDER BEI DEN KOHLENSTOFFVERBINDUNGEN. EINE PHARMAKOLOGIE DER KOHLENSTOFFVERBINDUNGEN BEKANNTER KONSTITUTION. 892 p. Berlin.
- (9) RICHARDSON, C. H., and GRIFFIN, E. L.
1926. MODIFICATIONS OF METHODS FOR MAKING COLD-MIXED OIL EMULSIONS. Jour. Econ. Ent. 19: 525-529.
- (10) ——— and SMITH, C. R.
1923. STUDIES ON CONTACT INSECTICIDES. U. S. Dept. Agr. Bul. 1160, 16 p.
- (11) RINGHARDTZ, H. [Ringhartz, K. J.]
1895. BEITRAG ZUR KENNTNIS DER WIRKUNG DES METANIKOTIN. 21 p. Kiel. (Inaug. Diss.) [Not seen.]

- (12) TATTERSFIELD, F.
1927. THE RELATIONSHIP BETWEEN THE CHEMICAL CONSTITUTION OF ORGANIC COMPOUNDS AND THEIR TOXICITY TO INSECTS. *Jour. Agr. Sci.* 17: 181-208, illus.
- (13) ——— and GIMINGHAM, C. T.
1927. STUDIES ON CONTACT INSECTICIDES. PART V. THE TOXICITY OF THE AMINES AND N-HETEROCYCLIC COMPOUNDS TO APHIS RUMICIS L. *Ann. Appl. Biol.* 14: 217-239, illus.
- (14) ——— GIMINGHAM, C. T., and MORRIS, H. M.
1925. STUDIES ON CONTACT INSECTICIDES. PART I. INTRODUCTION AND METHODS. PART II. A QUANTITATIVE EXAMINATION OF THE TOXICITY OF TEPHROSIA VOGELII HOOK. TO APHIS RUMICIS L. (THE BEAN APHIS). *Ann. Appl. Biol.* 12: 61-76, illus.
- (15) GIMINGHAM, C. T., and MORRIS, H. M.
1925. STUDIES ON CONTACT INSECTICIDES. PART III. A QUANTITATIVE EXAMINATION OF THE INSECTICIDAL ACTION OF THE CHLOR-, NITRO- AND HYDROXYL DERIVATIVES OF BENZENE AND NAPHTHALENE. *Ann. Appl. Biol.* 12: 218-262, illus.



DEVELOPMENT OF COTTON FIBERS IN THE PIMA AND ACALA VARIETIES¹

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INTRODUCTION

The spinning value of cotton fibers is dependent in large measure on their length and degree of maturity. The thickness of fiber walls is closely correlated with degree of maturity and fiber strength. Other fiber characters influence the spinning quality of cotton, but those mentioned are of major importance and are the ones which were studied in this particular investigation.

The extent to which factors external to the cotton plant, such as soil and climatic conditions, influence fiber length and maturity is not well understood. A knowledge of the life history of cotton fibers from the flowering of the plants, at which time fiber growth begins, to the maturity of the fibers is necessary in order to establish the degree to which external factors influence the length of fibers and the thickness of the fiber walls. The only published information relative to the development of cotton fibers which has come to the author's attention is the work of Balls² in Egypt. His material was taken from a single series in which the flowers opened near the beginning of the flowering period. The variety used was a pure strain of Egyptian No. 77 which has a fiber length of about 30 mm. as determined by measurements taken on combed fibers attached to the seed. Balls found that the fibers increased in length until the twenty-fifth day after flowering. The maximum rate of growth in length occurred near the fifteenth day after flowering. Thickening of the fiber walls began about 21 days after flowering, reached its maximum rate of increase in 36 to 39 days, and was practically completed 45 days after flowering.

The data included in the present publication relate to the life history of two types of cotton fibers developing in successive periods during the growing season of 1926.

MATERIAL AND METHODS

Pima, the only variety of American-Egyptian cotton now grown in the Southwest, has a fiber length of approximately 40 mm. ($1\frac{1}{2}$ to $1\frac{3}{4}$ inches). Acala cotton is the upland variety most widely grown in the Southwest, and has a fiber length of 28 to 30 mm. ($1\frac{1}{4}$ inches). These two varieties were chosen for the work on fiber development because they are representative of the two types of cotton grown commercially in the Southwest.

The fiber material studied was obtained from Acala and Pima plants growing on adjacent plots at the Salt River Valley Experiment Station. The plants were irrigated with sufficient frequency to prevent

¹ Received for publication Aug. 6, 1929; issued May, 1930.

² BALLS, W. L. THE DEVELOPMENT AND PROPERTIES OF RAW COTTON, p. 73-85, illus. London, 1915.

abnormal water stress but not so frequently as to produce overgrown plants. The soil in these plots is a fertile clay loam with a moisture equivalent of approximately 19.

Several hundred flowers of each variety were tagged on July 13, August 3, August 24, and September 14, making four series at 3-week intervals. A study of Figure 1 shows that the July 13 series began near the fore part of the flowering period and the September 14 series near the close of the flowering period. The August 3 and August 24 series flowered during mid season when flowering was at its height. Six bolls were collected from each series at 3-day intervals from flowering to maturity and stored in 5 per cent formalin for later study.

DEVELOPMENT OF FIBER LENGTH

Three measurements were made of the length of the wet fibers in each boll immediately after the bolls were taken from the preserving

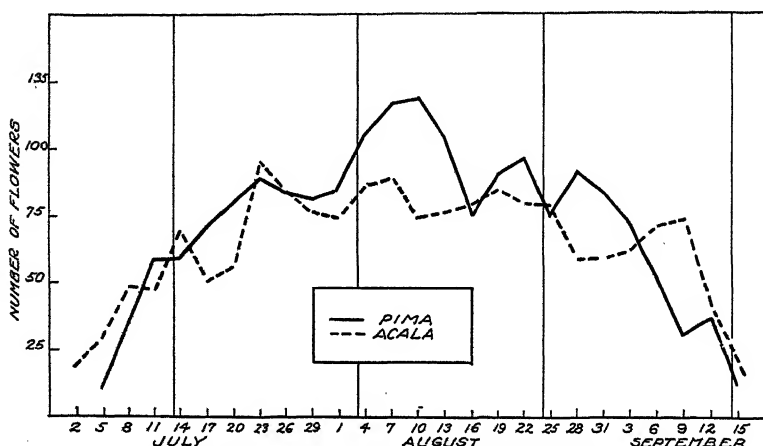


FIGURE 1.—Rate of flowering on 25 plants of the two cotton varieties studied

solution. Thus 18 measurements were made for each 6-boll collection. The fibers in the 3-day-old bolls were too short to measure satisfactorily without the aid of a microscope, but succeeding measurements were made without one.

INCREASE IN FIBER-WALL THICKNESS

Several hundred fibers were taken from each boll and infiltrated and embedded in paraffin in preparation for sectioning. (Celloidin and gelatin are also used for the infiltration of textile fibers with certain advantages over the paraffin method.) The embedded material was sectioned with a microtome having a drawing cut. Considerable difficulty was experienced in cutting the young fibers before the walls began to thicken, but as the walls thickened the fibers were more easily sectioned. The sections were mounted with albumen glycerin and stained with gentian violet.

The rate of wall thickening is relatively slower than the increase in fiber length, and it was soon found that measurements taken on material collected at 6-day intervals furnished dependable information on this development.

RESULTS

Cotton fibers are unicellular elongations from the epidermal layer of the ovule. The fibers begin to extend beyond the epidermis almost immediately after fertilization and are plainly visible under proper magnification 24 hours later. A comparison of Figures 2, 3, and 4 shows the rapidity of fiber growth following fertilization. Some fiber growth occurs even in ovules in which fertilization has been prevented by the removal of the anthers before the flower has opened. Fiber growth in unfertilized ovules is much slower for the first three

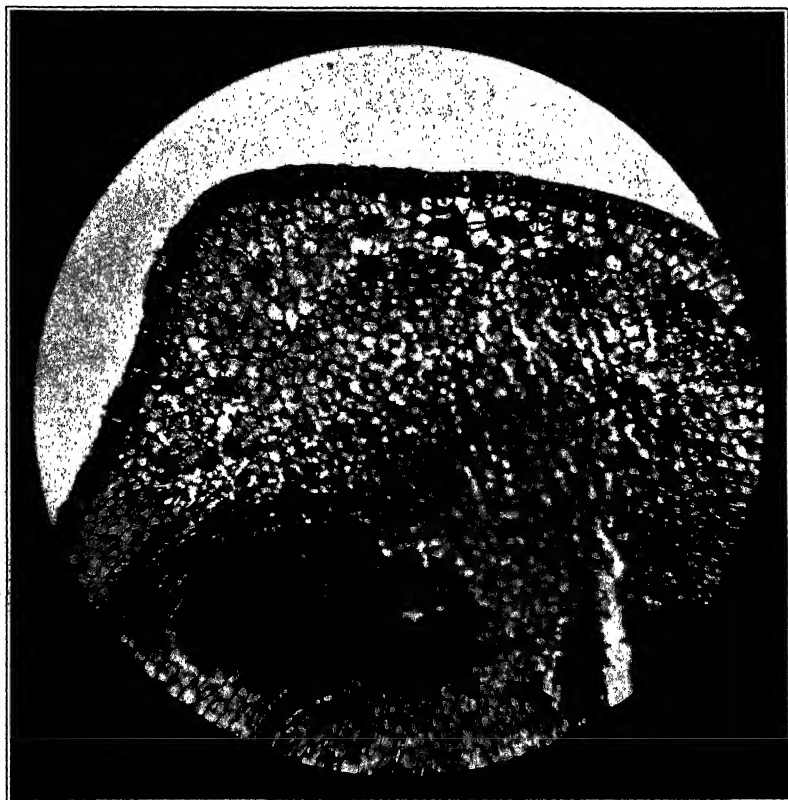


FIGURE 2.—Cross section of cotton ovule showing appearance of epidermis on day of fertilization. No transformation of epidermal cells into fibers is evident. Highly magnified

days than in fertilized ovules, and very little if any growth takes place after the third day, abscission of the boll usually occurring within seven or eight days after flowering.

The cumulative increase in fiber length of both Acala and Pima cotton is shown in Figure 5 and in Tables 1 and 2. Increase in length was practically completed in the first three series of Acala fibers in 21 days and in the corresponding series of Pima fibers in 27 days after flowering. The September 14 series of each variety required three days longer for the completion of fiber elongation, or 24 and 30 days for Acala and Pima, respectively. It will be noted that the temperatures were declining rapidly during the time when

the September 14 series of Acala and Pima fibers were developing length. The minimum temperatures had dropped considerably below 60° F., and the maximum temperatures had declined at least 10°. Pima cotton required about six days longer than Acala to complete growth in fiber length due largely to the fact that the Pima fibers were 10 mm. longer than the Acala. Dry fibers from mature bolls were used for the final measurements in each series; wet fibers from immature green bolls were used for all other measurements. This accounts for the drop at the end of each curve.

The curves showing the cumulative increase in fiber length in each series of Pima cotton have been superimposed in Figure 6 and those

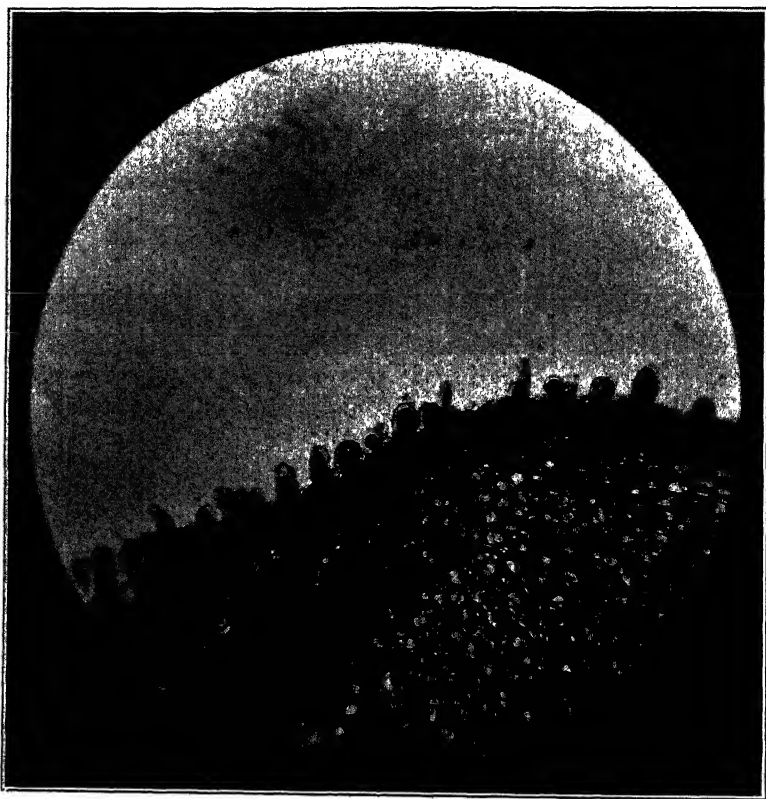


FIGURE 3.—Cross section of cotton ovule showing beginning of fiber growth from epidermal layer 24 hours after fertilization. Highly magnified

of Acala in Figure 7. These figures show that prevailing conditions did not create any great differences between either the Acala or the Pima series in the increase in length.

The rate of increase in fiber length is shown in Figure 8. The rate of fiber elongation was materially greater with Acala fibers than with Pima fibers after the ninth day from the flowering period. The most rapid increase occurred three days earlier with the Acala fibers than with the Pima in the first two series and six days earlier in the last two series. The greatest increase in fiber length was about 8

mm. in three days, the Pima fibers making slightly more rapid growth than the Acala in the first three series and a full millimeter more in the September 14 series. This means that these cotton fibers were making a daily growth in length of from three-thirty-seconds to one-eighth inch at the time of their most rapid growth. The temperatures which prevailed during the development in fiber length did not appear to cause any material differences in the rate of growth in the first three series but prolonged the period of growth in the September 14 series.

The curves showing the rate of fiber elongation have been superimposed in Figures 9 and 10. The most rapid increase in length of



FIGURE 4.—Cross section of cotton ovule showing development of fibers 48 hours after fertilization. Highly magnified

the Pima fibers occurred during the 3-day period ending on the twenty-first day after the flowering period in all series, as shown in Figure 9, while the most rapid increase in length of the Acala fibers occurred during the 3-day period ending on the fifteenth day in the August 24 and September 14 series and during the period preceding the eighteenth day in the July 13 and August 3 series, as shown in Figure 10. Present data do not indicate the factors which caused the retardation in the rate of fiber elongation from about the ninth to the fifteenth day in all of the Pima series and also in the Acala series to a lesser degree from the ninth to the twelfth day.

TABLE 1.—Development of fiber length in Pima cotton from flowering to maturity

Age of bolls (days)	Mean length of lint with \pm in—			
	July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series
	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>
3	0.4 \pm 0.041	0.4 \pm 0.050	0.3 \pm 0.047	0.3 \pm 0.028
6	2.9 \pm .543	3.0 \pm .159	2.7 \pm .222	2.4 \pm .960
9	7.1 \pm .667	6.7 \pm .299	6.1 \pm .331	5.8 \pm .455
12	11.9 \pm .597	11.0 \pm .704	10.9 \pm .554	10.0 \pm .450
15	17.0 \pm .959	16.4 \pm .712	15.2 \pm .471	13.3 \pm .654
18	24.4 \pm 1.635	23.2 \pm .424	22.8 \pm .361	19.8 \pm .705
21	32.4 \pm .880	31.7 \pm 1.043	31.1 \pm .390	28.8 \pm .575
24	39.1 \pm .882	37.5 \pm .915	37.8 \pm .522	35.6 \pm 1.204
27	40.5 \pm 1.355	39.7 \pm .829	39.2 \pm .876	38.5 \pm 1.140
30	40.4 \pm .782	40.1 \pm .527	39.7 \pm .533	39.9 \pm .812
33	40.6 \pm .749	40.3 \pm .657	39.8 \pm .719	39.9 \pm .470
36	40.8 \pm .875	40.1 \pm .524	39.9 \pm .314	39.9 \pm .805
39	41.4 \pm .518	40.0 \pm .566	39.9 \pm .555	40.2 \pm .500
42	40.4 \pm .676	40.2 \pm .601	40.0 \pm .471	40.4 \pm .755
45	40.9 \pm 1.053	40.2 \pm .381	40.3 \pm .463	40.0 \pm .666
48	41.0 \pm .499	40.2 \pm .533	40.0 \pm .745	40.1 \pm .424
51	40.7 \pm .650	40.2 \pm .533	40.1 \pm .705	40.1 \pm .458
54	37.5 \pm .590	40.4 \pm .558	40.1 \pm .705	40.3 \pm .443
57		37.9 \pm .741	40.3 \pm .666	40.1 \pm .458
60			39.9 \pm .404	40.1 \pm .404
63			36.9 \pm 1.282	40.5 \pm .678
66				40.1 \pm .229
69				40.3 \pm .652
72				40.1 \pm .458
75				40.1 \pm .566
78				40.1 \pm .404
81				40.3 \pm .471
84				38.7 \pm 1.609
Average ratio of mean length to standard deviation	40.50	51.73	57.35	63.60

TABLE 2.—Development of fiber length in Acala cotton from flowering to maturity

Age of bolls (days)	Mean length of lint with \pm in—			
	July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series
	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>
3	0.6 \pm 0.033	0.7 \pm 0.048	0.6 \pm 0.065	0.6 \pm 0.055
6	3.5 \pm .236	3.2 \pm .818	3.2 \pm .206	3.0 \pm .348
9	7.8 \pm .500	6.5 \pm .579	7.3 \pm .883	7.2 \pm .499
12	13.3 \pm .628	13.2 \pm .858	11.5 \pm .833	12.2 \pm .533
15	20.7 \pm .943	19.5 \pm .490	19.6 \pm .755	20.2 \pm .785
18	28.3 \pm .926	27.9 \pm .432	26.9 \pm .911	25.3 \pm .458
21	30.0 \pm .705	30.1 \pm .681	29.7 \pm .533	28.5 \pm .500
24	30.0 \pm .779	29.8 \pm .552	29.8 \pm .415	30.1 \pm .524
27	29.9 \pm .657	29.9 \pm .755	29.9 \pm .524	30.1 \pm .404
30	29.7 \pm .448	29.9 \pm .547	29.7 \pm .577	29.7 \pm .558
33	30.1 \pm .524	29.7 \pm .496	29.9 \pm .737	29.7 \pm .779
36	30.0 \pm .624	29.9 \pm .585	29.7 \pm .658	29.9 \pm .524
39	30.0 \pm .577	29.9 \pm .514	29.7 \pm .869	30.3 \pm .810
42	30.1 \pm .737	29.9 \pm .657	29.8 \pm .533	29.8 \pm .566
45	30.0 \pm .823	30.0 \pm .666	30.0 \pm .471	19.3 \pm .816
48	26.0 \pm 1.328	30.1 \pm .458	29.9 \pm .657	30.2 \pm .415
51		29.9 \pm .566	29.7 \pm .309	30.3 \pm .833
54		27.8 \pm 1.771	27.5 \pm 1.300	29.9 \pm .810
57				29.8 \pm .975
60				30.0 \pm .745
63				30.1 \pm .524
66				30.1 \pm .565
69				30.2 \pm .500
72				29.8 \pm .785
75				28.4 \pm 1.458
Average ratio of mean length to standard deviation	36.53	40.89	39.47	41.86

The standard deviation from the mean fiber length was exceptionally small with both varieties, as indicated in Tables 1 and 2. Deviations were much greater with the young fibers from 3 to 18 days old, probably due to the greater likelihood of error in measurements taken on short fibers as compared with those taken on the longer and older

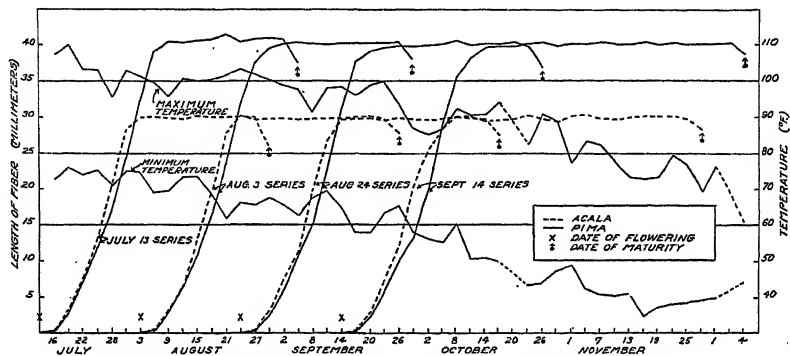


FIGURE 5.—Cumulative increase in fiber length in Acala and Pima cotton

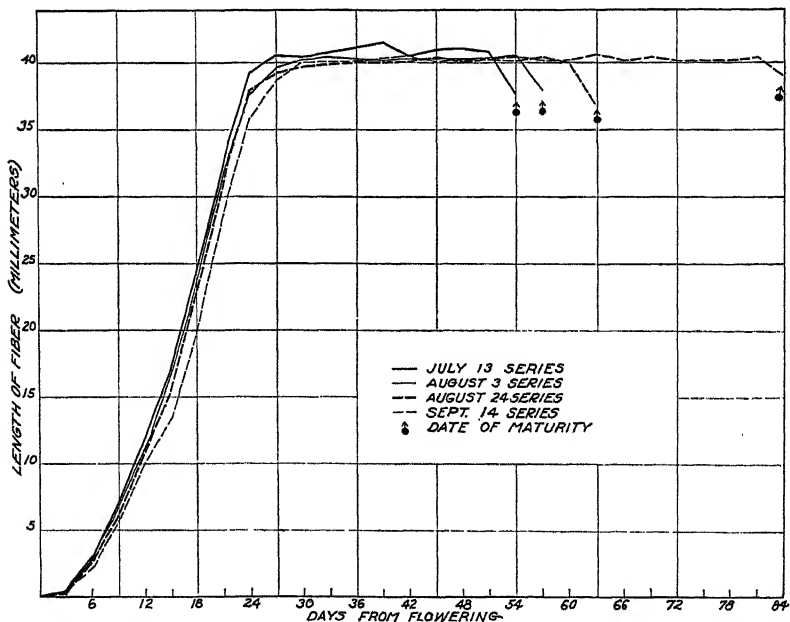


FIGURE 6.—Superimposed curves showing cumulative increase in length of Pima cotton fibers

fibers. The Pima fibers were more regular in length than were the Acala fibers, as indicated by the smaller deviations in length measurements in the former variety. This greater regularity of the Pima fibers became more pronounced as the season advanced, which is evidenced by the wider ratios between mean fiber lengths and the standard deviations in each successive series of the two varieties.

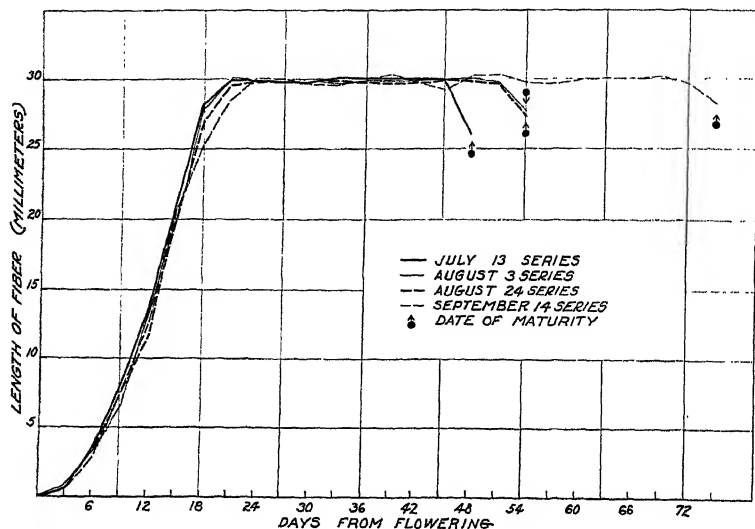


FIGURE 7.—Superimposed curves showing cumulative increase in length of Acala cotton fibers

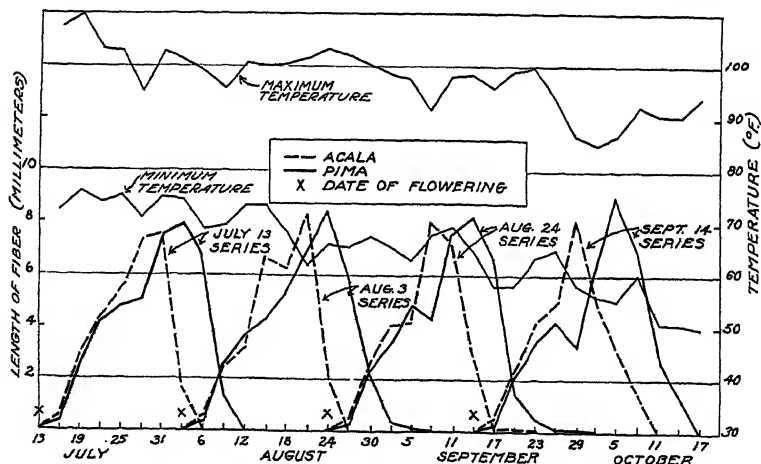


FIGURE 8.—The rate of increase in cotton fiber length as affected by temperature

TABLE 3.—Development of fiber-wall thickness in Pima cotton from flowering to maturity

Age of bolls (days)	Thickness of fiber wall in—				Age of bolls (days)	Thickness of fiber wall in—			
	July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series		July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series
6.....	Microns	Microns	Microns	Microns	57.....	Microns	Microns	Microns	Microns
12.....	0.30	0.35	0.35	0.40	60.....		2.80		
18.....	.32	.35	.35	.40	63.....			2.50	2.51
24.....	.35	.35	.38	.40	66.....			2.40	
30.....	.90	.70	.43	.40	68.....				2.79
36.....	1.85	1.02	.85	.51	72.....				2.65
42.....	2.40	1.90	1.70	.81	75.....				
48.....	2.75	2.55	2.00	1.55	78.....				2.80
54.....	2.80	2.90	2.37	1.80	84.....				2.80
	3.10	2.80	2.35	2.56					

TABLE 4.—Development of fiber-wall thickness in Acala cotton from flowering to maturity

Age of bolls (days)	Thickness of fiber wall in—				Age of bolls (days)	Thickness of fiber wall in—			
	July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series		July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series
6.....	<i>Microns</i>	<i>Microns</i>	<i>Microns</i>	<i>Microns</i>	57.....	<i>Microns</i>	<i>Microns</i>	<i>Microns</i>	<i>Microns</i>
12.....	0.36	0.40	0.35	0.40	60.....	-----	-----	-----	2.82
18.....	.35	.35	.35	.40	63.....	-----	-----	-----	-----
24.....	.36	.40	.35	.40	66.....	-----	-----	-----	2.83
30.....	.63	.70	.52	.52	72.....	-----	-----	-----	3.18
36.....	1.64	1.02	1.22	1.12	75.....	-----	-----	-----	3.20
42.....	2.28	2.25	1.65	1.43	78.....	-----	-----	-----	-----
48.....	2.58	2.50	2.00	1.82	84.....	-----	-----	-----	-----
54.....	2.75	2.80	2.70	2.10					
	-----	3.12	2.40	2.76					

The cumulative increase in fiber-wall thickness is shown in Tables 3 and 4 and Figure 11. Measurements were taken on material collected at 6-day intervals beginning six days after flowering and continuing until maturity. No appreciable thickening occurred during the first 18 days after flowering with either variety of cotton in any series. The fiber walls began to thicken sometime between the eighteenth and twenty-fourth day in the first two series and to a somewhat lesser extent in the third series. A small amount of thickening also took place in the September 14 series of Acala between the eighteenth and twenty-fourth day, but the walls of the pima fibers did not thicken until after the twenty-fourth day in this last series. The temperatures were declining rapidly during the time the fiber walls were thickening in the August 24 and September 14 series, the maximum temperatures dropping below 75° and the minimum below 40° before thickening was completed in the last series. These temperatures were evidently sufficiently low to inhibit the rapid thickening of the fiber walls. A comparison of Figure 5 with Figure 11 shows that during the period immediately succeeding flowering, elongation of the fibers was proceeding rapidly, but thickening of the fiber walls did not begin until elongation was almost completed. The rate of thickening was slow at first, but became considerably accelerated after elongation had been completed.

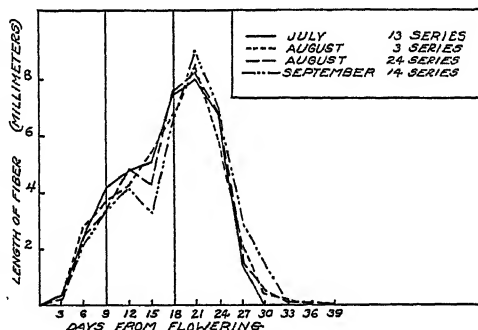


FIGURE 9.—Superimposed curves showing the rate of increase in length of Pima cotton fibers

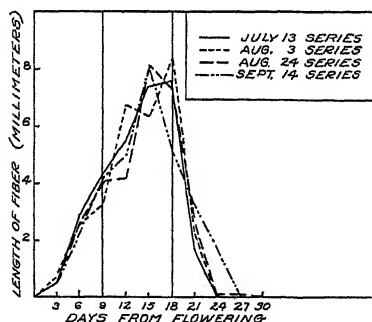


FIGURE 10.—Superimposed curves showing the rate of increase in length of Acala cotton fibers

proceeding rapidly, but thickening of the fiber walls did not begin until elongation was almost completed. The rate of thickening was slow at first, but became considerably accelerated after elongation had been completed.

The curves showing the development in thickness of fiber walls have been superimposed in Figures 12 and 13. Maximum fiber-wall

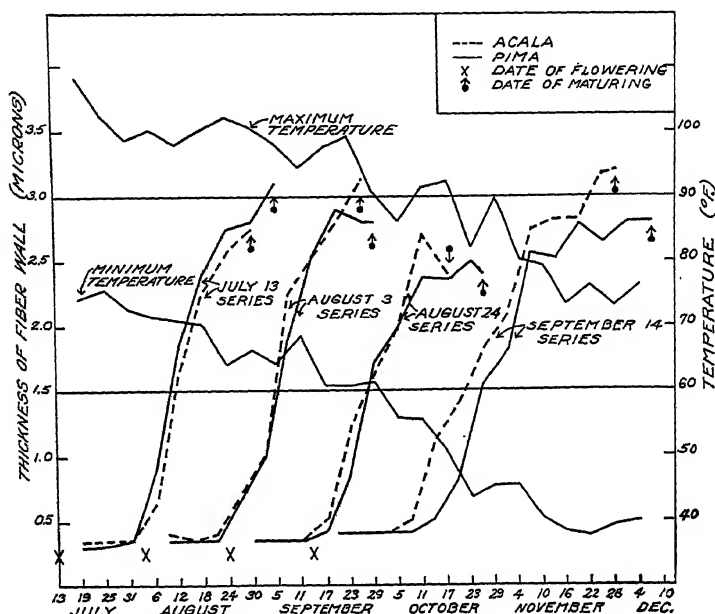


FIGURE 11.—Cumulative increase in fiber-wall thickness in Acala and Pima cotton

thickness was attained in the Pima variety 54, 48, 60, and 78 days after the flowering period in the July 13, August 3, August 24, and September 14 series, respectively. Acala cotton required 48, 54, 48,

and 75 days for maximum fiber-wall thickening in these respective series. With the exception of the August 3 series, the Pima fibers required from 3 to 12 days longer for wall thickening than did the Acala fibers.

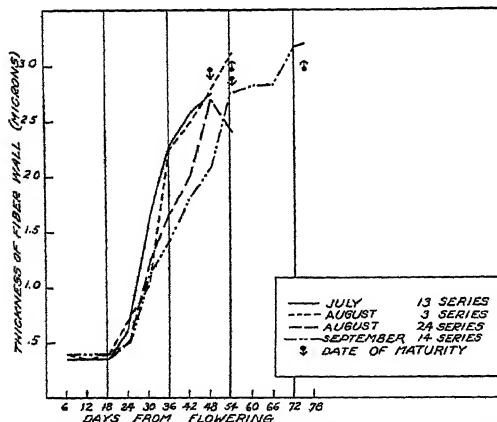


FIGURE 12.—Superimposed curves showing the cumulative increase in fiber-wall thickness of Pima cotton

the one exception of the August 3 series of Acala fibers. No explanation will be advanced as to why two high peaks with an intervening low rate of thickening occurred with the Pima fibers in the

The effect of temperature on the rate of increase in fiber-wall thickness is well shown in Figure 14. The rate of increase in thickness dropped as the temperatures declined with each successive series with

September 14 series and also in the August 24 and September 14 series of Acala fibers.

More than one-third of the total wall thickening of the first two series of Acala fibers was acquired during the 3-day period of most rapid thickening. Considerably less than this amount of wall thickening was acquired during the corresponding periods of the last two Acala series. The amount of wall thickening in the Pima fibers during the time of their most rapid thickening was somewhat less than for the Acala in the first two series and more than the Acala in the last two series.

The superimposed curves in Figures 15 and 16 emphasize differences in the rate of increase in fiber-wall thickness which prevailed between the various series. A comparison of these two figures with Figures 9 and 10 shows that differences between series in the rate of increase in fiber-wall thickness were much more pronounced than were

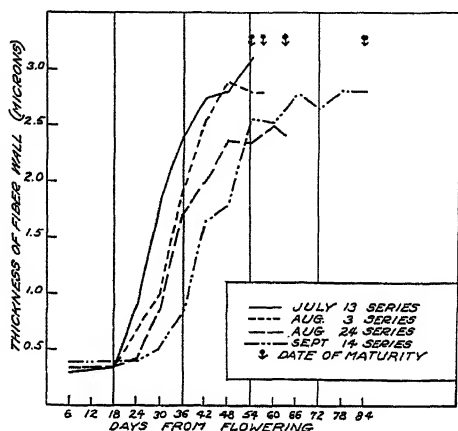


FIGURE 13.—Superimposed curves showing the cumulative increase in fiber-wall thickness of Acala cotton

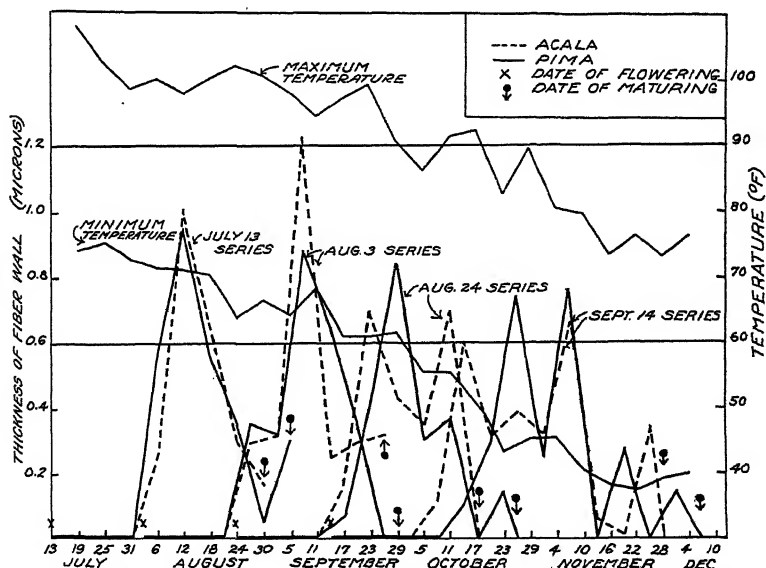


FIGURE 14.—The rate of increase in fiber-wall thickness in Pima and Acala cotton as affected by temperature

differences in the rate of fiber elongation. This was undoubtedly due to the fact that fiber elongation occurred first and was completed in most of the series before temperatures had declined to any great extent.

Fiber-wall thickening was completed at the date of maturity of the bolls in three of the Acala series and in one of the Pima series, while wall thickening was completed in the remaining series from three to nine days before the bolls opened. These differences were due in

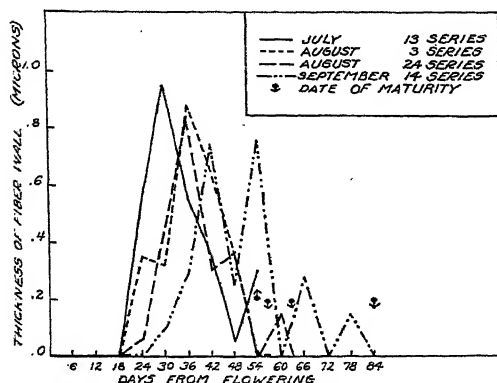


FIGURE 15.—Superimposed curves showing the rate of increase in fiber-wall thickness of Pima cotton

ceases within a few days in unfertilized bolls.

Elongation of Acala fibers was completed 21 days after flowering in the series which flowered July 13, August 3, and August 24; and 24 days after flowering in the series which flowered September 14.

Pima cotton required 27 days for the elongation of the fibers in the first three series and 30 days for those in the September 14 series.

Lower temperatures probably caused the prolongation of the time needed for completion of fiber length in the September 14 series of both varieties.

The fibers of both varieties made a daily increase in length of from three thirty-seconds to one-eighth inch at the time of their most rapid growth.

The greatest increase in fiber length occurred about the twenty-first day after flowering in the Pima series and from the fifteenth to the eighteenth day in the Acala series.

No appreciable thickening of fiber walls began until fiber elongation was almost completed.

The rate of fiber-wall thickening became less, with one exception, in each successive series as the temperatures declined.

Fiber-wall thickening was completed in the Pima fibers 54, 48, 60, and 78 days after the flowering period in the July 13, August 3,

part to the difficulty in deciding when a boll is mature. The tendency toward delayed opening of the bolls was greater during the latter part of the season and was probably due to the general slowing up of plant activity.

SUMMARY AND CONCLUSIONS

Fiber growth begins at the time of flowering irrespective of fertilization and proceeds rapidly after fertilization but

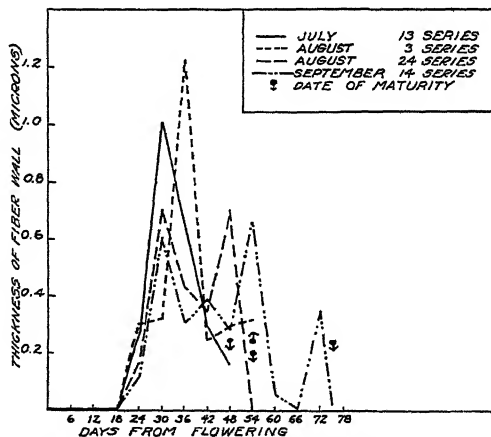


FIGURE 16.—Superimposed curves showing the rate of increase in fiber-wall thickness of Acala cotton

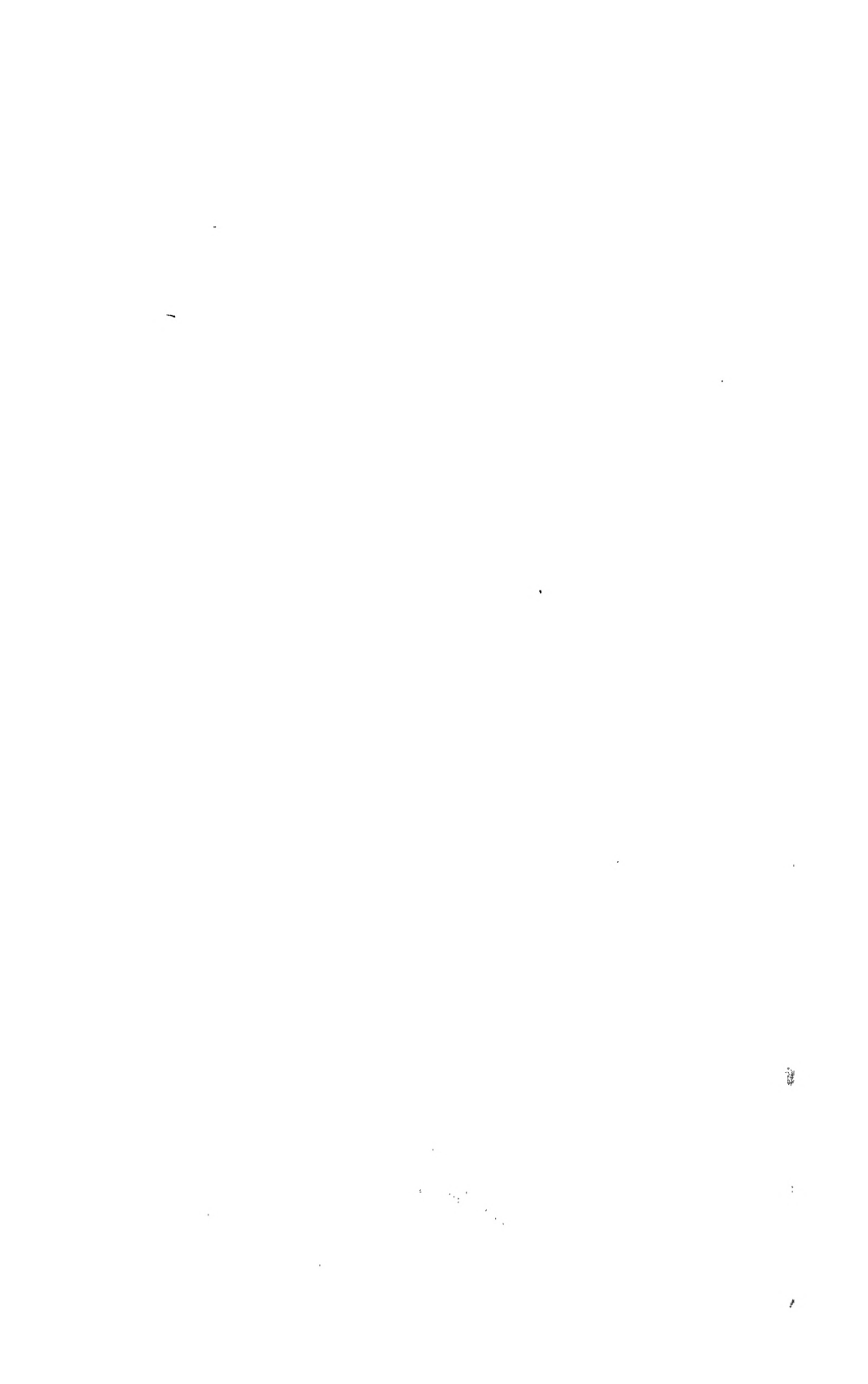
August 24, and September 14 series. Acala cotton required 48, 54, 48, and 75 days for maximum fiber-wall thickening in these series.

Fiber-wall thickening was completed in some instances at the time of boll maturity and in others a few days before maturity.

The time of the season during which cotton fibers are developing affects the rate of fiber-wall thickening greatly but does not influence the rate of fiber growth in length to any appreciable extent until late in the season.

Prevailing temperatures contribute to the rate of fiber development, and when lower than necessary for optimum plant growth, have a retarding effect on both fiber elongation and fiber-wall thickening.

Varietal differences in the development of length and wall thickness of cotton fibers as unlike as Acala and Pima are noteworthy.



SOURING OF FIGS BY YEASTS AND THE TRANSMISSION OF THE DISEASE BY INSECTS¹

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INTRODUCTION

Souring was the first disease of the fruit of the fig to be reported from California. For a long time it was the only disease recognized and the term was used indiscriminately to cover all kinds of spoilage. The first reference to this disease was made by Pierce, as reported by Galloway (11 p. 239-240⁴) in 1892. He states:

But another one of the industries of the State which has been greatly extended of late is seriously threatened. This is the growth and curing of figs. It has been observed since the cultivation of this fruit has been attempted that the grower had to contend with a destructive fermentation of the fruit which often caused the loss of nearly the entire crop.

Pierce found that the fruits spoiled, both while on the tree and on the drying trays and that the causal agent was a yeast.

Numerous experiments with powders and sprayers were used on the trees but with entirely negative results. The cause probably lies in the fact that the fruit is inoculated by insects, the yeast cells being carried by them to the ripening fruit.

No further results were published by Pierce. Howard (18, p. 93-94) in 1900 refers to souring in the Smyrna fig (*Calimyrna*) as follows:

Souring of the figs was not noticed in the early part of the season, but began later to a limited extent when showers occurred. When the Smyrna figs ripen the ostiolum opens wide and remains open so that a match can easily be inserted and often moderate-sized insects can enter and feed on the sugar. Some of them are caught in the sticky sap and die within the fig. When the figs are ripe and fall ants and beetles of the genera *Noctoxus* and *Carpophilus* enter in this way.

Roeding (27, p. 50) in 1903 studied the fig at home and abroad and mentioned souring as occurring in Asia Minor and in California.

Experience has shown, however, that the Smyrna varieties suffer far less from this trouble than the ordinary sorts. In the orchard of the Fancher Creek Nurseries, where a few of the White Adriatic figs are still growing, from 50 to 75 per cent will sour on the trees, and in adjoining rows of Smyrna figs it is only occasionally that a sour fig can be found.

Eisen (8) and Rixford (26) also mention souring in an inclusive way. Rixford found that a closed eye prevents fermentation. Coit and Johnston, as reported by Haring (17) in 1921, did not find a specific organism responsible for the decay of figs observed. "Many types of rot were observed, from the soft, watery, fermented type to the typical dry-rot type. Various yeasts, fungi, and bacteria seem to be responsible."

¹ Received for publication Nov. 5, 1929; issued May, 1930.

² Most of the work was done while the writer was the recipient of the James Rosenberg memorial scholarship in agriculture.

³ The writer wishes to acknowledge his indebtedness to Prof. Ralph E. Smith of the University of California, under whose direction the investigation was carried on, for suggestions and criticisms, and for critical reading of the manuscript.

⁴ Reference is made by number (italic) to Literature Cited, p. 1049.

The writer commenced work on this problem in the fall of 1922. It immediately became evident that the decay commonly given the name "souring" was not caused by one agent alone, and that the symptoms were obvious enough to allow the division into at least two distinct diseases. It was furthermore found that the symptoms on caprifig varieties, such as the Lob Ingir, Stanford, San Pedro Black, and several unnamed seedlings requiring caprification, were rather of the character of a rot than of a fermentation and were found solely on caprifig figs. While fermentation also occurred on caprifig



FIGURE 1.—Sour Adriatic fig with jellylike exudate coming out of the eye which indicates that active fermentation is taking place within the fruit

figs, alone or at the same time as the rot, figs of parthenocarpic varieties such as the Adriatic, Mission, Kadota (Dottato), and spring crop San Pedro Black, never exhibited the rot. The findings of the writer on this rot of the fig, termed "endosepsis," have already been discussed (5). It is the purpose of this paper to discuss the etiology and transmission of souring.

SYNONYMY AND SYMPTOMS

Souring, as previously mentioned, is the name commonly used to describe all forms of fig spoilage. The term should be restricted to the symptoms described below to cover the spoilage due to fermenta-

tion organisms. Fig fermentation would be a more appropriate name for this disease, but the term "souring" is well established and, when restricted to the disease under discussion, is not confusing.

The symptoms are best observed on fruit of parthenocarpic varieties that have not been caprifigged, such as the Adriatic. In Lob Ingir and other caprifigged figs the symptoms are liable to be obscured or confused with the symptoms of endosepsis, or internal rot, that attacks only caprifigged figs. The symptoms of the disease are manifested only when



FIGURE 2.—Interior of sour fig shown in Figure 1

the figs begin to ripen and the eye is wide open. In no case has any deterioration been observed in figs of parthenocarpic varieties before the eye opens. Philips et al. (23) and Caldis (5) have found that the pulp of such figs is sterile previous to the opening of the eye and for considerable time afterwards.

In souring there is at first a change in the color of the pulp, which from pink becomes colorless, and subsequently turns watery. A pink liquid exudes through the eye (fig. 1), dropping on the leaves or jelly-

ing at the eye. Gas bubbles are seen through the pulp and in the skin, and in many cases the pulp becomes water-soaked and loses its firmness. The pulp is disintegrated and smells strongly of alcohol (fig. 2), and is often found to be covered by a white scum. (Fig. 3.) In this condition the figs begin to shrivel and dry up, either dropping to the ground or hanging on the twig, in the latter case giving rise to what is commonly called "black neck" figs. A dead spot or "eye canker" is often formed in the bark at the point of attachment of such figs, as seen in the two twigs at the top of Figure 4. Fermented figs lose their firmness and sag, and usually the pulp becomes detached from the skin at the neck which shrivels, dries up, and turns dark.

Fig souring is primarily an alcoholic fermentation, but subsequent changes may take place while the fig is attached to the twig, on the ground or on the drying board. The commonest change is the action of acetic bacteria on the alcohol with the production of acetic acid,

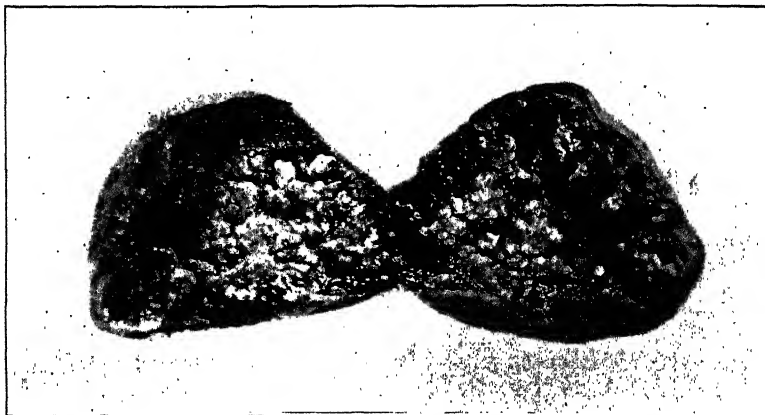


FIGURE 3.—Yeast scum sometimes found inside sour figs

which is readily discerned by its pungent, strong odor. Ethyl acetate and other esters are probably formed.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Souring has been observed wherever the fig is grown in California. No section is free from it, although orchards have been observed with a very small percentage of figs suffering from this disease. Estimates as to the percentage of injury can not be accurate in the case of Lob Ingirs on account of the coexistence of endosepsis; however, in the case of Adriatics, which are not caprifried, souring is very abundant in certain seasons and certain localities and at times increases the cull pile to include the entire crop. There have been cases known to the writer where the crop has not been gathered at all on account of this disease. A grower of Adriatics in the San Joaquin Valley estimated the loss from souring in 1923 as 80 per cent. Such losses are common. Indeed, Adriatic figs seldom escape a high percentage of spoilage from this cause. The reasons for variations from year to year and from locality to locality can best be discussed when the facts regarding transmission have been given. The influence of

the environment will be taken up also at the time, as well as varietal susceptibility.

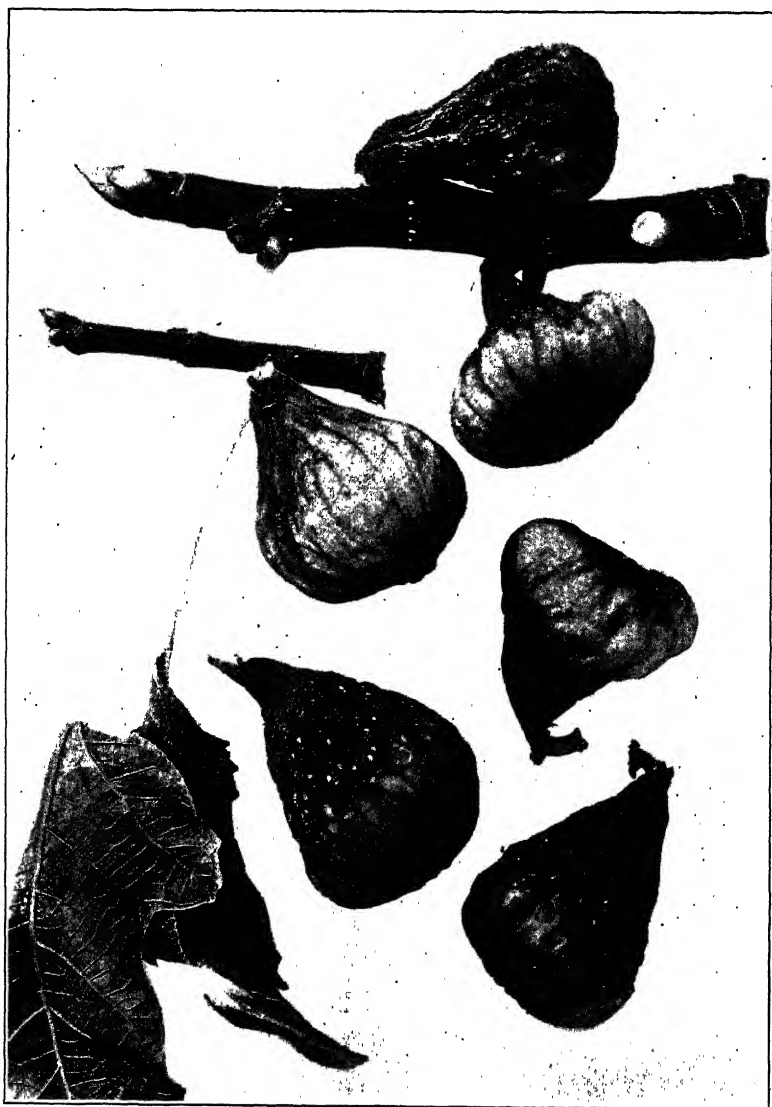


FIGURE 4.—Dried, sour, "black neck" figs, some of which are still attached to twig; note also dead spot or "eye canker" formed in the bark at point of attachment of such figs.

This disease has been observed in foreign fig-growing countries by several travelers, but there is no reference in literature as to importance and extent of damage. Letters of inquiry were written to the departments of agriculture of Italy, France, Greece, Spain, Jugoslavia, Portugal, Turkey, and South Africa in the fall of 1922.

Replies were received only from Italy, Spain, and South Africa. G. B. Traverso, director of the Royal Station of Vegetable Pathology, Rome, Italy, stated that the disease "does not exist in Italy." The director general of agriculture and forestry of Spain replied that "the disease probably exists in Spain * * * but there have been few figs seen with these characteristics, and they never constitute an epidemic." I. Tribolet, of the division of horticulture, department of agriculture, Union of South Africa, stated that he had never seen the disease in that country.

No mention of souring is made by Edgerton (7), Gould (12), or Matz (21) in their studies of fig diseases in the South Atlantic and Gulf States. Siniscalchi (32), De Rosa (28), Portale (24), and Guglielmi (13) make no mention of souring among the fig diseases enumerated as occurring in Italy. Ferrari (10), writing about the fig industry in Cosenza, Italy, describes a disease of the fruit in connection with a bacteriosis of the tree. He states that the figs attacked by the disease when nearly ripe show a drop of liquid at the eye, first yellow, then reddish, which increases in volume and, if the infection is heavy, is exuded. This may be souring, but there are no definite data given. Vallese (36) also mentions a disease of the fig in Italy that may be similar to souring. Trabut (35) and Guillochon (14) from North Africa and Esterlich (9) from Spain do not mention souring at all in their papers.

Condit, in an unpublished report on the fig industry in Europe, Asia Minor, and north Africa, states the following about fig souring:

In my report on my trip to Europe I find the following regarding fig souring: The fact undoubtedly is that fig souring occurs more commonly than the Europeans like to admit. The fancy Smyrna layer or Locoum figs, the Greek string figs, or the Spanish fleur figs, which are seen in city markets, represent only the best part of the crop. For example, fig merchants of Smyrna prefer the crop from the hillside orchards of the upper Meander Valley because the figs in the Sokia district and especially in the Ayassouluk or coastal district are soft, sour, dark colored, and inferior. It is a well-known fact that the largest proportion of the fig crop of Mallorca Province, Spain, is used for hog feed or distilled on account of its poor quality. The packers of Coin, Malaga Province, Spain, state that the growers deliver only 50 per cent of the crop to the packing house, feeding the other inferior half to cattle and hogs. Immense quantities of sour and rotten or inferior figs are shipped from Southern Italy to Trieste or Vienna for coffee factories. I myself found Smyrna figs souring at Ayassouluk on August 20, but not in serious quantities. At Kalamata figs of a garden variety were souring badly in the yard of Mr. Pantaxopoulos near the seashore on September 4. Rain-damaged figs were very abundant throughout southern Europe this season, but these could hardly be classed as sour figs. The lateness of the season in Spain and Portugal prevented actual observations along this line.

ETIOLOGY

FORMS OF THE YEASTS ISOLATED

Pierce (11, p. 240) isolated

a yeast, which when pure cultures were made, was applied to fruit on both trees and the drying board. The result was the production of an exactly similar fermentation to that occurring naturally.

No identification of the organism is reported. Coit and Johnston (17) could not assign a specific organism to the decay. They found various yeasts, fungi, and bacteria apparently responsible for the disease.

When fruit not affected with endosepsis was examined and showed the symptoms described previously, especially the early ones, the

author has invariably isolated pure cultures of yeasts. The disease was studied mostly on figs of the Adriatic variety. This variety is very susceptible to souring, it is widely planted in California and, when it is not caprified, endosepsis does not affect it. There is more than one species of yeast responsible for this disease. It is conceivable that every true yeast introduced into the cavity of the fig, when the latter is ripe, the cavity full of sugary solution and the concentration not too high, would be capable of exciting fermentation. In many platings of sour Adriatics, however, it was found that principally two forms of yeasts were invariably present. A third one was also present but was found incapable of producing the disease. All three forms belong to the class of asporogenous or wild yeasts. The form or forms most often found is a top yeast that produces a scum in liquid media and in the fig cavity and belongs most probably to the *Mycoderma* class. (Fig. 5.) The second form often present is a bottom yeast, growing poorly on artificial media, especially liquid ones, and belonging to the *Apiculate* class. The third form is a round yeast of the *Torula* type. These forms are hereafter designated as A (*Mycoderma*), C (*Apiculate*), and E (*Torula*). Single-cell isolations of these yeasts made by the micropipette method were used in inoculations and in studying their fermentation and other physiological activities.

CULTURAL CHARACTERISTICS OF THE YEASTS ISOLATED

TWO PER CENT DEXTROSE BROTH

Yeast A: A thin veillike pellicle is produced on the surface of the liquid in 24 hours and climbs up the sides of the tube. The liquid is cloudy with an abundant sediment. Copious gas is evolved on shaking the tube.

Yeast C: A scarcely noticeable precipitate is formed at the bottom of the tube. This yeast grows very poorly, if at all, in the liquid media used, except in grape juice. A brownish pigment is produced at room temperature (21° C.) and at 28° C. No pigment at lower temperature.

Yeast E: Cloudiness and abundant precipitates are produced, but no pellicle. A small amount of gas produced by some strains.

TWO PER CENT DEXTROSE NUTRIENT AGAR

Yeast A: An abundant, spreading, dull white, somewhat powdery growth is produced, the margin is lobate, with ciliate edge more evident on plates. (Fig. 5.) The center of the stroke is raised, whereas the margins are filmlike.

Yeast C: Very scanty, wet, transparent, beaded growth is produced, with a brownish pigment at room temperature.

Yeast E: Abundant, spreading, white, shiny butyrous growth, with smooth margin.

MALT AGAR⁵

Yeast A: Abundant growth, dull white, less than on dextrose nutrient agar.

Yeast C: Scanty whitish growth.

Yeast E: Abundant shiny white growth.

⁵ One hundred grams of germinated barley was ground up and 1,000 c.c. of water added. The mixture was heated at 55° to 58° C. for one hour and held over for 24 hours. It was then boiled and filtered, agar added, was tubed and sterilized.

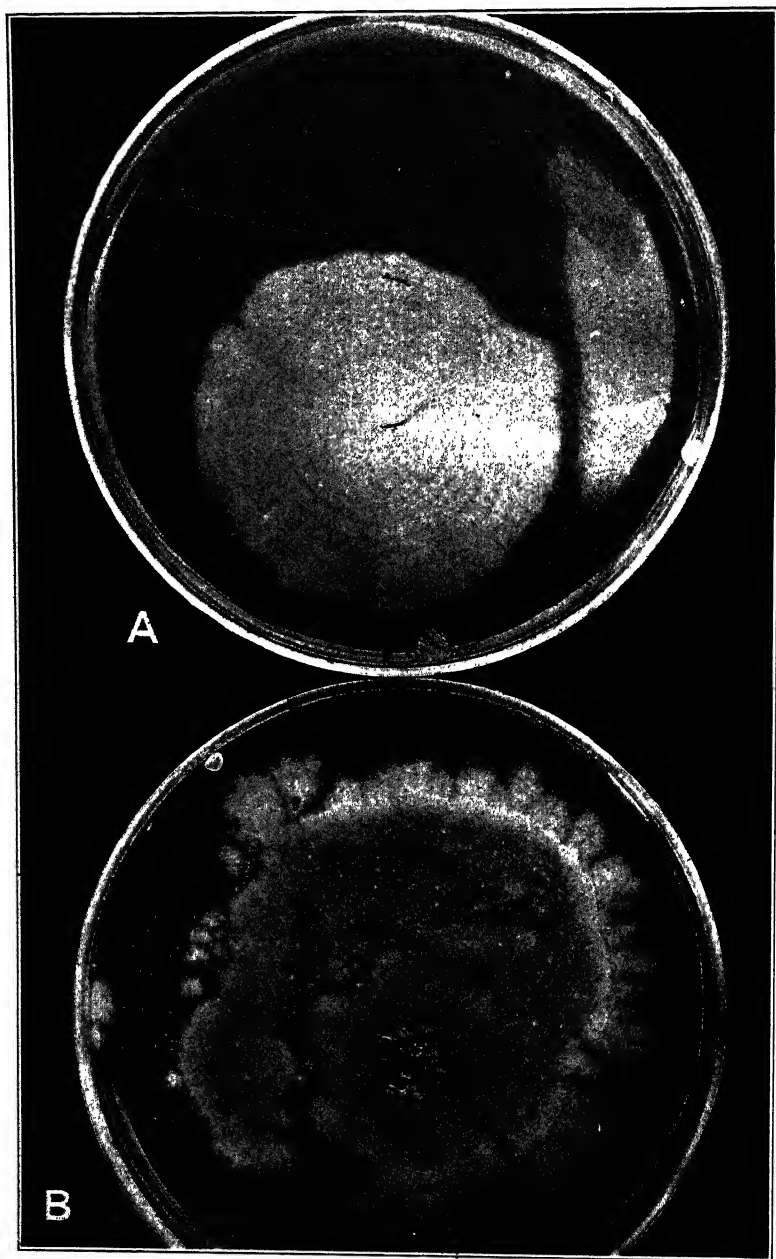


FIGURE 5.—Cultures of yeast A obtained (A) from dried-fruit beetle and (B) from the internal tissue of a sour fig

FOUR PER CENT DEXTROSE LAURENT LIQUID¹

Yeasts A and E: As in dextrose bouillon.

Yeast C: No growth.

GIANT COLONIES ON TWO PER CENT DEXTROSE NUTRIENT AGAR PLATE

Yeast A: Growth rapid, irregular, dull white, with rough surface, effuse elevation, margin lobate, cilliate. (Fig. 5.)

Yeast C: Slow growth, round to irregular, with flat, smooth surface, undulate to lobate edge.

Yeast E: Medium growth, round colony, three-fourths of an inch in diameter, white, shiny, elevation effuse, surface ringed, edge entire.

LAURENT AGAR PLATE

Yeast A: Large irregular colonies, dull white.

Yeast C: Small transparent white colonies.

Yeast E: Small, shiny white round colonies.

FIG INFUSION⁷

Yeast A: Thin pellicle, liquid clear, brownish sediment, thick ring.

Yeast C: Slight ring, no pellicle, brown sediment.

Yeast E: As in A.

FERMENTATION OF SUGARS BY THE YEASTS ISOLATED

Glucose, fructose, sucrose, lactose, and maltose have been tested, as well as apple cider, grape juice, and fig infusion. The sugars were added either to beef bouillon, to Laurent solution, or to the ammonium phosphate medium used in sugar fermentation with bacteria (33). Smith and Dunham fermentation tubes were used.

Nine isolations of yeast A, three of yeast E, and four of yeast C were inoculated into Smith fermentation tubes containing 2 per cent dextrose, fructose, sucrose, maltose, lactose bouillon, cider, Zinfandel grape juice, and fig infusion.

It is evident from Table 1 that the disaccharides are not fermentable by the three yeasts isolated from figs. Gas was produced from dextrose and fructose to a great extent by yeast A. Yeast C produced a small amount of gas from fructose 30 days after inoculation. Yeast E also produced a small amount. Gas was produced abundantly by the three yeasts from grape juice, and by yeasts A and C from fig infusion, but not by yeast E. Pellicle, a cloudy bulb, but a clear arm was produced by yeast A on all the above media. Maximum gas was produced by yeast A on all the media. Maximum gas was produced in 10 to 12 days. The nine isolations of yeast A produced variable percentages of gas from dextrose in the closed arm of the fermentation tube. The percentages varied from 4.7 to 23.5 per cent, an average of 14.4 per cent produced in from 5 to 8 days. The greater percentage of gas was produced in 8 days, the next (23.5 per cent) in 5 days, while the lowest amount of 4.7 per cent was produced in 10 days.

⁶ Composed of 4.71 gms. ammonium sulphate, 0.75 gm. potassium phosphate, 0.1 gm. magnesium sulphate, 1,000 c. c. water and 2 per cent of the sugar to be studied.

⁷ One hundred grams of dried figs boiled in 1,000 c. c. of water for one-half hour, filtered and sterilized. Agar added, if wanted.

TABLE 1.—Results of fermentation tests of various sugars and fruit juices with yeasts obtained from sour figs

Medium	pH	Yeast A	Yeast C	Yeast E
2 per cent dextrose broth	6.4	Abundant gas.	Slight growth, no gas.	Small amount gas, slight growth.
2 per cent fructose broth	6.4	do.	Slight amount in 30 days.	No gas.
2 per cent sucrose broth	6.6	No growth.	No growth.	Small amount gas.
2 per cent maltose broth	6.5	do.	do.	No growth.
2 per cent lactose broth	6.5	do.	do.	Do.
Apple cider	3.4	Small amount gas.	do.	Do.
Zinfandel grape juice	3.9	Abundant gas.	Abundant gas.	Abundant gas.
Fig infusion	4.8	do.	do.	No gas.

Zinfandel grape juice was found to be a favorable medium for all these yeasts. To test their fermentation abilities, 200 c. c. of grape juice was placed in 500 c. c. Erlenmeyer flasks and sterilized by steaming for three consecutive days. They were then inoculated in duplicate with three single cell isolations of yeast A made by the micropipette method, one of yeast C, and one of yeast E. The flasks were weighed daily, and when the loss of weight was comparable to that of the check, a portion of 100 c. c. was distilled with the addition of 50 c. c. of water. The three strains of yeast A produced an average of 7.10 per cent of alcohol by volume (4.7 per cent by weight). Yeast C produced 8.22 per cent and yeast E 6.55 per cent (6.6 and 5.5 per cent, respectively, by weight).

For another test of the fermentation and acid production by yeasts A and E, ammonium phosphate medium and beef-extract broth, both solid and liquid, with the addition of brom cresol purple indicator, were used with each of the following sugars: Dextrose, sucrose, and lactose. The solid media were slanted, the liquids were put in Dunham fermentation tubes. Yeast A produced both acid and gas from the dextrose synthetic, and gas but no acid from the dextrose bouillon. Neither acid nor gas was produced from either the liquid or solid, the synthetic, or the bouillons with the addition of sucrose and lactose. Yeast E produced acid on the liquid synthetic dextrose, but no gas. A small amount of gas, but no acid, was produced after 13 days from dextrose bouillon. Acid and gas were produced from sucrose, both in the synthetic and the broth media. Neither acid nor gas was produced from lactose in either type of medium. The production of gas by yeast E was found to be irregular. Of the three isolations made, which otherwise appear identical, one produced small amounts of gas from glucose and grape juice, the other two did not.

As mentioned previously, the yeasts isolated from figs act indifferently toward disaccharides. It is of interest that figs do not contain such sugars. Leclerc du Sablon (30) found very little non-reducing sugars in the varieties Dorée (Figue d'or), Datte Quodidienne (Figue datte), and Barnissotte Black (Bourjasotte noir). An analysis for sugars of healthy and diseased Adriatic figs was made by the writer in 1923. Healthy figs picked fresh contained 69.61 per cent of reducing sugars soluble in alcohol, while sour figs, picked as they dropped from the tree, contained 59.45 per cent of reducing sugars soluble in alcohol, both percentages calculated on a moisture-free basis. No nonreducing sugars were found in either the healthy or the diseased figs.

To demonstrate that this disease is actually a fermentation, the following experiment was tried: Sour Adriatic figs were selected from

the drying board and examined for the typical symptoms of the disease. Eight hundred grams of the figs were macerated with 500 c. c. of water and steam-distilled for three hours. The first portion of the distillate was neutralized with potassium hydroxide and redistilled. Fifty seven cubic centimeters of distillate was obtained, with a specific gravity of 0.9812, or 13 per cent of alcohol by volume. The second fraction was also neutralized and redistilled, 250 c. c. was obtained, with a specific gravity of 0.9950, or 2.20 per cent of alcohol by volume. Sour Adriatic figs picked from the ground as they were dropping from the trees, were treated as above and 3.55 per cent of alcohol by volume was obtained. A peculiar, ethereal odor passed into the distillates.

Rossi (29), in his extensive review of the literature regarding apiculate yeasts, mentions that *Pseudosaccharomyces apiculatus* isolated from grapes produces 3.15 per cent of alcohol by weight from dextrose. He states that acetic and formic acid as well as esters and other volatile substances were found by Amthor, Müller-Thurgau, Kayser, Seifert, and Mach e Portele to be produced by this yeast. It has been mentioned already that sour figs at times have an ester smell.

MORPHOLOGY OF THE YEASTS ISOLATED

Yeast A: A top yeast with long and narrow cells varying considerably in length. Budding apical, the daughter cells remaining attached to form long chains with as many as three daughter cells attached to the same apex. A few cells are slightly curved. The cells are vacuolated, especially on solid media. No spores are produced on plaster blocks, Gorodkova's medium (20) or carrot plugs. The cells are 6 to 33 by 2.2 to 6 μ (average 8.9 by 3.9) on dextrose nutrient agar and 7.5 to 31.5 by 3 to 6 μ (average 12.4 by 4.7) on dextrose bouillon. (Fig. 6.)

Yeast C: Typically apiculate bottom yeast measuring 4.1 by 2.0 μ , single or in pairs, sometimes elliptical, never in threads. No spores found. (Fig. 6.)

Yeast E: A bottom yeast with almost perfectly round cells, with a large vacuole, budding freely, the daughter cells remaining attached for some time forming chains of three or four cells. No spores are produced on plaster blocks, Gorodkova medium or carrot. Budding cells measure 4.19 by 3.4 μ and 3.9 by 3.9 μ when not budding. Some strains are smaller, measuring 3.1 by 3.1 μ . (Fig. 6.)

PATHOGENICITY OF THE YEASTS ISOLATED

Inoculations with single-cell cultures of these yeasts were made on fresh figs that were still attached to the tree. Figs were selected with the eye fairly well closed and inoculated by inserting a needle through the eye.

The whole twig was then bagged with a 3-pound manila paper bag and tied firmly in order to exclude insects from the fig. (Fig. 7.) Inoculations were made in triplicate. The eyes of the figs inoculated were previously swabbed with alcoholic mercuric chloride. The figs when ripe were brought to the laboratory and plated.

Yeast A: Typically sour figs were obtained by inoculating with this yeast. The pulp of the figs was discolored and covered by a white scum. One side of the fig was softened and water-soaked. The figs were often bloated, gassy, and sagging, with the pulp drawn away from the stem end. Slight dripping was observed. When green figs were inoculated, the results were sometimes negative.

Yeast C: Fermented figs were obtained with this yeast essentially as with yeast A, but without the scum on the surface of the pulp cavity.

Yeast E: Inoculations with this yeast did not yield typically sour figs. The pulp of the fig was discolored and gelatinized with an odor approaching that of sour figs.

TRANSMISSION

Berlese (2, 3, 4) has found that yeasts are very scarce in the air during late spring and early summer, but abundant on trunks and

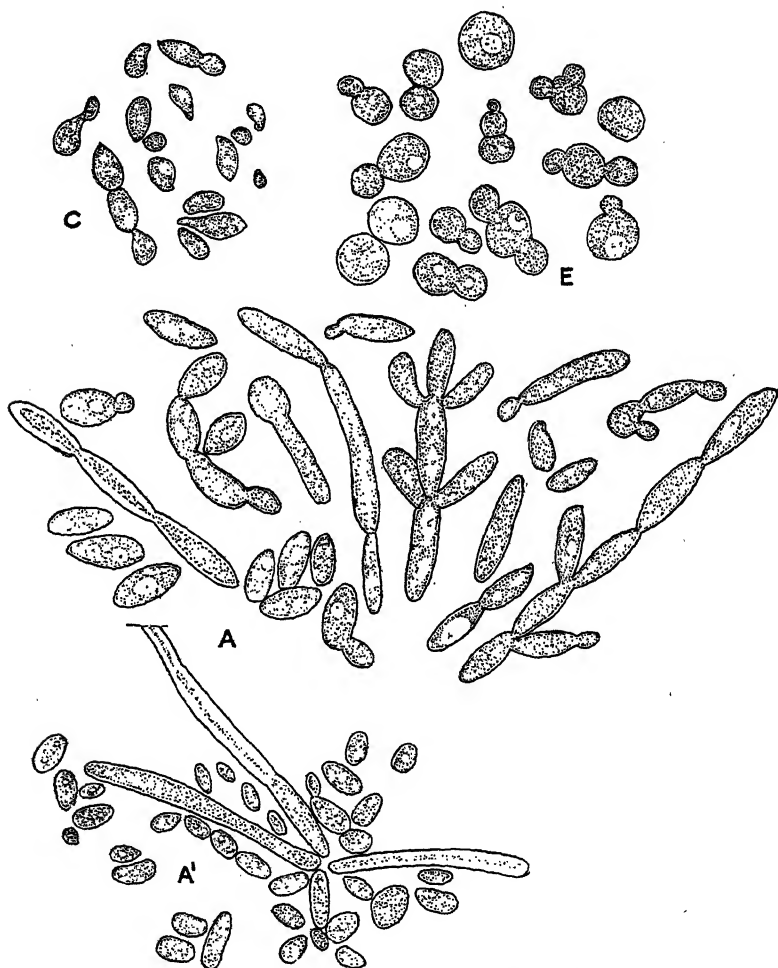


FIGURE 6.—Yeasts isolated from sour figs. Camera lucida drawings $\times 1,400$ from 7-day old cultures. Yeasts A and E grown on Laurent liquid, C on dextrose nutrient agar. A', pellicle of yeast A on dextrose bouillon

other parts of trees. He found *Saccharomyces apiculatus*, *S. ellipsoideus*, and *S. pastorianus* in the soil from April to June, but rarely afterwards. Insects, however, such as ants and flies (*Sarcophaga carnaria* L., *Drosophila cellaris* L., *Calliphora erythrocephala*, *Dasyphora*, *Crysotoxum*, *Eristalis*, *Aricia*, *Lucilia*, and *Anthomyia* sp.),

were found carrying yeasts both externally and internally. Yeasts were found to multiply in their intestines and to hibernate in them.

Phillips et al. (23) in their studies on fig smut found that exclusion of insects from two large fig trees by building a tent of muslin cloth over the trees reduced the amount of souring to nothing, while there was a large number of sour figs on the neighboring trees. They also found that the insects most common in figs still on the tree were the dried-fruit beetle, *Carpophilus hemipterus* L., and the vinegar fly, *Drosophila ampelophila* Löw. It may be deduced, therefore, that these two insects may be concerned in the transmission of the yeasts causing souring into the cavity of the fig. In order to test this theory, a number of sacks were made of brass strainer cloth 1 by 1.5 feet,

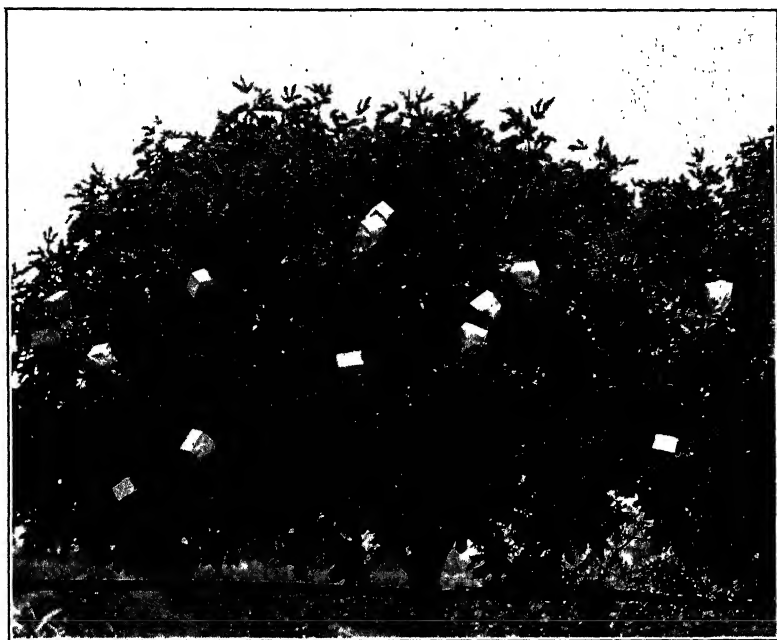


FIGURE 7.—Figs covered with paper bags to exclude insects in experiments on the transmission of souring by inoculation with yeasts

soldered on top and side. These sacks were slipped over fig twigs bearing from 3 to 9 figs, fully grown but green, whose eye scales were tightly closed. The sack was sewed at the bottom, and a large plug of cotton placed in the opening through which the twig was introduced. One hundred manila paper sacks were also placed on similar figs. (Fig. 7.) Adriatic figs were used in this experiment in 1924; six strainer-cloth sacks were placed on August 1 and six more on August 26. When the figs commenced ripening in the sacks (September 8) 12 dried-fruit beetles, collected from figs in sterile vials by holding the mouth of the vial over the eye of the fig and tapping the sides so that the beetles emerged from the fig and entered the vial, were introduced into each of four of the sacks. The beetles were watched awhile, and they were seen crawling on the figs. Many sour figs were on the

trees on which the sacks were placed. When every fig in the sacks ripened and dried up, the twigs bearing the sacks were cut and brought to the laboratory for examination. The results are given in Table 2.

TABLE 2.—Effect upon souring of exclusion and introduction of *Carpophilus hemipterus* L. into strainer-cloth sacks inclosing ripening Adriatic figs

No beetles introduced			12 beetles introduced in each sack				
Sack No.	Total figs	Sour	Sack No.	Total figs	Sour	Wormy but not sour	Sound
1.....	5	None.	4.....	7	3		3
2.....	4	None.	6.....	9	3	6	
3.....	9	None.	7.....	5	2		3
5.....	8	None.	12.....	5		5	
8.....	4	None.	Total.....	26	8	11	6
9.....	6	None.					
10.....	3	None.					
11.....	6	None.					
Total.....	45	0					

The beetles fed and bred in many of the figs, as many more beetles were found in the sacks than the number introduced. Larvae were also found in them. No insects of any kind were found in the eight sacks where they were not purposely introduced. The results indicate that the beetles enter the figs readily but do not cause fermentation unless they are themselves infected. In many cases the wormy figs had been sour, but the alcohol had evaporated in drying, leaving a seedy, dry fig, wormy, devoid of sugar but not sour in the ordinary sense. This has been observed also in the orchard. As mentioned previously, the spoilage may stop at any stage in its development, depending on drying conditions, moisture, and presence of the proper organism.

The beetles from the different sacks were collected and cultured in tubes of dextrose bouillon. The sour figs were plated. The results are as shown in Table 3.

TABLE 3.—Results of making cultures from *Carpophilus hemipterus* L. found in sacks containing sour figs

[Cf. Table 2]

Sack No.	Number of beetles	Number infected with yeasts	Type of yeast
4.....	3	1	C
6.....	8	3	A
7.....	7	7	C
12.....	8	0	-----

From these experiments it may be concluded that *Carpophilus hemipterus* L. is a transmitting agent of yeasts and bacteria into the figs, where they set up fermentation if the sugar concentration is favorable. To determine the actual flora carried by the dried-fruit beetles, and especially whether the transmission is internal or external, beetles were collected at different times from fig orchards by holding

the mouth of a sterile vial against the eye of an infested fig and tapping the sides. The beetles entered the vial and were thus taken to the laboratory alive and uncontaminated. *Drosophila* were caught in the same way. The insects were studied as follows: (1) Caught with sterile tweezers and dropped into sterile tubes of dextrose bouillon. One beetle was thrown into each tube. The beetles were either crushed or dropped according as they were dead or alive; (2) placed in Gooch crucibles and disinfected by immersing in 15 per cent solution of potassium hydroxide for 2 minutes, then in 1:500 solution of mercuric chloride for 3 minutes, and washed finally with sterile (autoclaved) water until the washings were free of chlorides as determined by testing with silver nitrate solution; momentary dipping in 95 per cent alcohol was at times used, instead of potassium hydroxide, and 1:1,000 alcoholic (50 per cent) solution of mercuric chloride, instead of the 1:500 solution. No difference was observed in the two methods of disinfection, both being equally effective. After disinfection, the beetles were dropped into tubes of sterile dextrose bouillon, one beetle per tube, left in this tube for varying lengths of time, 1 to 20 minutes, and then removed by means of a sterile platinum loop and dropped into another tube where they were left. The purpose of the shaking into the first tube for varying lengths of time was to obtain a check on the effectiveness of the external sterilization. Unsterilized beetles were shaken into one tube first and transferred into a second tube afterwards in order to determine whether there was any difference between their external and internal flora. Different lots of beetles were tested separately, and beetles were also collected from decaying watermelons, which serve as overwintering grounds for these insects, as was found by Phillips et al. (23) and the writer. The results of these tests are summarized in Tables 4 and 5.

TABLE 4.—Results of various culture tests on *Carpophilus hemipterus* L., sterilized and unsterilized, as a carrier of yeasts

UNSTERILIZED

[Figures refer to numbers of insects cultured]

Treatment of beetles	Carpophilus hemipterus L. secured from—													
	Figs							Watermelons						
	Aug. 30, 1924	Sept. 12, 1924	Oct. 6, 1924	Oct. 30, 1924	Nov. 11, 1924	Oct. 27, 1925	Nov. 5, 1925							
Type of yeast														
	A	C	Neg- ative	A	C	Neg- ative	A	C	Neg- ative	A	C	Neg- ative	A	C
Dropped in bouillon and left for 1 to 19 minutes, then removed	2		1	9	3	1	4	8	13	2	4	3	4	9
Insects from last	14	2	2	4	2	1	1	1	2	1	1	1	1	2
STERILIZED														
Dropped in bouillon and left (uncrushed)	5		5	5	3	3					3	6	1	3
Dropped in bouillon and left (crushed)	4	6	6	4	2	3						8	2	2
Left for 1 to 20 minutes then removed (shaken)			5		9	9						10		10
Insects from last	4		2	2	5	5					3	13	2	6

* The insects were sterilized after removing from tube 1.

TABLE 5.—*Summary of Table 4*

UNSTERILIZED							
Item	Beetles carry- ing yeast A		Beetles carry- ing yeast C		Beetles nega- tive		Total beetles used
	Number	Per cent	Number	Per cent	Number	Per cent	
1. Dropped in bouillon and left.....	31	26	30	26	56	48	117
2. Left for 1 to 19 minutes, then removed.....	19	53	5	14	12	33	36
3. Insects from 2.....	3	21	6	43	5	36	14
STERILIZED							
1. Dropped in bouillon and left (uncrushed)....	70	16	10	23	26	61	43
2. Dropped in bouillon and left (crushed).....	9	15	10	16	42	69	61
3. Left for 1 to 20 minutes, then removed (shaken).....					75	100	75
4. Insects from 3.....	8	10	10	13	59	77	77

The fig tissues yielded the usual yeasts, yeast C and yeast A. In sack 12, where none of the figs soured, the beetles were found to be free of yeasts. Different bacteria were also obtained from the beetles. The figs inclosed in manila paper sacks at the same time as the strainer cloth sacks were also found to be in perfect condition.

This experiment was repeated in 1925 at Davis, Calif., figs of the following varieties being used: Adriatic, Vernal, and Mission, using 20 sacks, which inclosed a total of 147 figs. Of these, 29 figs (3 sacks) were exposed to dried-fruit beetles. Nine of them were found wormy and on plating yielded the usual yeast. The figs were dry and seedy, smelling faintly of alcohol. The remaining 20 figs were not sour. The sacked figs which were not exposed to beetles (17 sacks, 118 figs) were all found to be in perfect condition.

Rand and Pierce (25) in 1920 have reviewed the literature on the subject of insect transmission of plant and animal diseases. Insects transmit pathogenes in three ways: (1) Mechanically, by picking up the spores on the exterior of their bodies and accidentally sowing them on the surface or inoculating them into punctures; (2) by making avenues of infection through wounds; and (3) by transmitting them internally, either mechanically or biologically.

The results in Table 4 show that both forms of yeasts isolated from fermenting figs are carried by the dried-fruit beetle. When the beetles were dropped in tubes of broth and left for different intervals of time, 1 to 10 minutes, and then removed, fermentation was set up in many cases (67 per cent), indicating that the types of yeasts mentioned previously are carried mechanically externally. The elytra, the legs, the thorax, and the abdomen were removed and tested separately, and were all found carrying the yeasts. Fermentation was also caused by beetles dropped into bouillon tubes and left uncrushed or crushed. When the beetles were previously sterilized by one of the methods described and then dropped into tubes of bouillon and left uncrushed or crushed, fermentation was also set up. When such beetles were shaken for a limited length of time, 1 to 20 minutes, in a tube of broth and then transferred to another tube where they were left crushed or even uncrushed, there was never fermentation or any kind of growth in the first tube, but in many cases (23 per cent) fermentation was set up in the second tube, indicating that transmission is also internal mechanical. No attempt was

made to discover whether the transmission is internal biological, i. e., whether the yeasts actually multiply in the intestines of the beetles. Berlese (4) has shown this to be the case with flies. Both types of yeasts were found to be carried at the same time, and in one case one yeast was found carried externally and the other internally.

As the season advanced a larger number of beetles were found not carrying yeasts. Different bacteria and especially a short rod in chains were usually associated with the yeasts or obtained as the sole flora. *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp. were also obtained from beetles, the first by far the greatest number of times.

The opinion has been frequently expressed that vinegar flies, *Drosophila* sp., are attracted to fermenting figs but do not themselves enter healthy ones and so spread the disease. The writer has observed them to enter or issue from figs which when examined afterwards were apparently healthy. Vinegar flies were caught in the same way as dried-fruit beetles and were found carrying the yeasts both externally and internally.

While the flies are attracted by the odors of fermentation and are known to feed on yeasts and decaying fruit, as reported by Baumberger (1), Schulze (31), Sturtevant (34), and Northrop (22), Baumberger has found that adult *Drosophila* flies oviposit on sterile fruit, the presence of yeasts being unnecessary, and Guyénot (15, 16) has raised *Drosophila ampelophila* aseptically for two years (40 generations), indicating that *Drosophila* flies may enter sound fruit for the purpose of breeding and feeding and in so doing transmit the fig-souring organisms.

Phillips et al. (23) in their studies of the transmitting agents of *Aspergillus niger* causing what is called black smut in figs consider *Carpophilus hemipterus* first in importance and *Drosophila ampelophila* as second. The habitats of the dried-fruit beetle throughout the year are discussed. The writer has made observations which confirm the results obtained by Phillips. The appearance of the first sour figs in many orchards coincided with the appearance of the first beetles, and in frequent trips through the figs district of the State isolated orchards have been studied for the presence of both the beetles and souring. It seemed that wherever decaying fruit, melons, oranges, figs, apples, apricots, peaches, plums, etc., were left on the ground, offering a breeding place for the beetles and the vinegar fly, souring was invariably abundant. Young Adriatic fig orchards in sections where grain farming predominated were found free of souring and beetles. It was interesting to note the souring of the first crop figs of Adriatic, Brunswick, Mission, Kadota, and White San Pedro. These come early in the season (June) when the overwintering generation of the beetle has not multiplied extensively; souring, therefore, is found only in such figs in orchards with decaying oranges, melons, or other fruits having imperfect sanitation. In connection with orchard sanitation and the presence of souring, an inquiry was made by I. J. Condit about the presence of the dried-fruit beetle in Italy where souring is reported as nonexistent. Doctor Briganti, of Portici, Italy, wrote that "*Carpophilus hemipterus* is rarely found in Italy * * * cases of fig smut and the manifestation of the fruit beetle are so sporadic and of so slight an importance as not to interest deeply our entomologists." In a populous country like Italy, fruits decaying under the trees or on the drying grounds are very scarce.

The idea has been often expressed by Eisen (8), Coit (6), Coit and Johnston (17), and is prevalent among growers that cold nights, overirrigation, cool damp weather, or other environmental conditions cause souring. In view of the evidence presented above, moisture variations can have no other than a modifying effect. Given the infection, carried by the dried-fruit beetle or the vinegar fly, slow drying of the fig with the accompanying slow increase in sugar concentration may favor greatly the development of the parasite. The opposite would be the case with quick drying. The yeasts would probably never develop or their activities would be quickly checked. To find out the effect of high sugar concentration on the growth of yeasts, nutrient agar was prepared to which 40 and 60 per cent of dextrose were added, instead of the customary 2 per cent. The yeasts grew readily on 40 per cent but made very poor growth on the 60 per cent dextrose agar.

SUMMARY

A destructive fermentation of figs, both caprifig and parthenocarpic, is described, with a discussion of the economic importance of the disease and its geographical distribution.

Three different types of yeasts were isolated from fermenting figs; their cultural characteristics, fermentation ability, morphology, and pathogenicity are discussed.

The transmission of these yeasts into the cavities of figs by the dried-fruit beetle (*Carpophilus hemipterus*) is shown. The transmission was found to be both internal and external mechanical.

LITERATURE CITED

- (1) BAUMBERGER, J. P.
1919. A NUTRITIONAL STUDY OF INSECTS WITH SPECIAL REFERENCE TO MICROORGANISMS AND THEIR SUBSTRATA. Jour. Expt. Zool. 28: 1-81, illus.
- (2) BERLESE, A.
1897. RAPPORTI FRA LA VITE ED I SACCAROMICETI. MEMORIA I-II. SULLA DISTRIBUZIONE DEI FERMENTI ALCOOLICI NELLA NATURA. Riv. Patol. Veg. 5: 211-237, 263-282, 354-360, illus.
- (3) ———
1897. RAPPORTI FRA LA VITE ED I SACCAROMICETI. MEMORIA III. RICERCHE SUI MEZZI DI TRASPORTO DEI FERMENTI ALCOOLICI. Riv. Patol. Veg. 5: 295-341, illus.
- (4) ———
1898. RAPPORTI FRA LA VITE ED I SACCAROMICETI. MEMORIA IV. SOPRA GLI HABITAT INVERNALI DEI FERMENTI ALCOOLICI. Riv. Patol. Veg. 6: 1-20.
- (5) CALDIS, P. D.
1927. ETIOLOGY AND TRANSMISSION OF ENDOSEPSIS (INTERNAL ROT) OF THE FRUIT OF THE FIG. Hilgardia 2: [287]-328, illus.
- (6) COIT, J. E.
1921. FIG SPLITTING AND SOURING CAN BE REDUCED. Pacific Rural Press 101: 300.
- (7) EDGERTON, C. W.
1911. DISEASES OF THE FIG TREE AND FRUIT. La. Agr. Expt. Sta. Bul. 126, 20 p., illus.
- (8) EISEN, G.
1901. THE FIG: ITS HISTORY, CULTURE, AND CURING. WITH A DESCRIPTIVE CATALOGUE OF THE KNOWN VARIETIES OF FIGS. U. S. Dept. Agr., Div. Pomol. Bul. 9, 317 p., illus.
- (9) ESTERLICH, P.
1910. LA HIGUERA Y SU CULTIVO EN ESPAÑA. 228 p. Barcelona, Spain.

- (10) FERRARI, E.
1912. LA COLTIVAZIONE DEL FICO NEL CIRCONDARIO DI PAOLA (COSENZA). MEMORIA MONOGRAFICA. Ann. R. Staz. Sper. Agrumic. e Fruttic. Acireale 1: [141]-177.
- (11) GALLOWAY, B. T.
1893. REPORT OF THE CHIEF OF THE DIVISION OF VEGETABLE PATHOLOGY. INVESTIGATIONS OF THE SPECIAL AGENT IN CALIFORNIA. U. S. Dept. Agr. Sec. Rpt. 1892:238-241.
- (12) GOULD, H. P.
1919. FIG GROWING IN THE SOUTH ATLANTIC AND GULF STATES. U. S. Dept. Agr. Farmers' Bul. 1031, 47 p., illus.
- (13) GUGLIELMI, G.
1908. COLTIVAZIONE INDUSTRIALE DEL FICO NEL LECCESE.—MEMORIA MONOGRAFICA. Bol. Arbor. Ital. 4:11-18, 57-65, 114-125, 152-156.
- (14) GUILLOCHON, L.
1913. TRAITÉ PRATIQUE D'HORTICULTURE POUR LE NORD D'AFRIQUE. FIGUIER. p. 263-270. Tunis.
- (15) GUYÉNOT, E.
1913. ÉTUDES BIOLOGIQUES SUR UNE MOUCHE, DROSOPHILA AMPELOPHILA LÖW. I.—POSSIBILITÉ DE LA VIE ASEPTIQUE POUR L'INDIVIDU ET LA LIGNÉE. Compt. Rend. Soc. Biol. Paris 74:97-99.
- (16) ———
1913. ÉTUDES BIOLOGIQUES SUR UNE MOUCHE, DROSOPHILA AMPELOPHILA LÖW. II.—RÔLE DES LEVURES DANS L'ALIMENTATION. Compt. Rend. Soc. Biol. Paris 74: 178-180.
- (17) HARING, C. M.
1922. SPLITTING AND SOURING OF SMYRNA FIGS. Calif. Agr. Expt. Sta. Ann. Rpt. 1920/21:80-81.
- (18) HOWARD, L. O.
1901. SMYRNA FIG CULTURE IN THE UNITED STATES. U. S. Dept. Agr. Yearbook 1900:79-106, illus.
- (19) KAYSER, E.
LES LEVURES. Paris.
- (20) MANEVAL, W. E.
1924. A METHOD OF SECURING SPORES OF YEASTS. Bot. Gaz. 78:122-123.
- (21) MATZ, J.
1918. SOME DISEASES OF THE FIG. Fla. Agr. Expt. Sta. Bul. 149, 11 p., illus.
- (22) NORTROP, J. H.
1917. THE RÔLE OF YEAST IN THE NUTRITION OF AN INSECT (DROSOPHILA). Jour. Biol. Chem. 30:181-187.
- (23) PHILLIPS, E. H., SMITH, E. H., and SMITH, R. E.
1925. FIG SMUT. Calif. Agr. Expt. Sta. Bul. 387, 38 p., illus.
- (24) PORTALE, F.
1910. LA COLTIVAZIONE DEL FICO NEL CIRCONDARIO DI MISTRETTA. MEMORIA MONOGRAFICA. Bol. Arbor. Ital. 6:51-101, illus.
- (25) RAND, F. V., and PIERCE, W. D.
1920. A COÖRDINATION OF OUR KNOWLEDGE OF INSECT TRANSMISSION IN PLANT AND ANIMAL DISEASES. Phytopathology 10:[189]-231.
- (26) RIXFORD, G. P.
1918. SMYRNA FIG CULTURE. U. S. Dept. Agr. Bul. 732, 48 p., illus.
- (27) ROEDING, G. E.
1903. THE SMYRNA FIG AT HOME AND ABROAD. 87 p., illus. Fresno, Calif.
- (28) ROSA, F. DE
1911. DI ALCUNI FICCHI SALENTINI. Atti R. Ist. Incoragg. Napoli (6) 9:1-36.
- (29) ROSSI, G. DE
1920. I LIEVITI APICULATI NELLA FERMENTAZIONE VINARIA. Staz. Sper. Agr. Ital. 53:233-297, illus.
- (30) SABLON, L. DU
1908. OBSERVATIONS SUR LES DIVERSES FORMES DU FIGUIER (FICUS CARICA). Rev. Gén. Bot. 20:129-150, 207-216, illus.
- (31) SCHULZE, P.
1911. ENTWICKLUNG VON DROSOPHILA RUBROSTRIATA BRECKER IN FORMOL; EIN BEITRAG ZUR KENNTNIS DER LEBENSWEISE DER DROSOPHILALARVEN. Zool. Anz. 39:199-202.

- (32) SINISCALCHI, A.
1912. LA COLTIVAZIONE DEL FICO NEL CILENTO (PROV. DE SALERNO).
MEMORIA MONOGRAFICA. Bol. Arbor. Ital. 7: 25-41, 49-54.
- (33) SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIAL
TECHNIC.
1926. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA . . .
46 p., illus. Geneva, N. Y.
- (34) STURTEVANT, A. H.
1916. NOTES ON NORTH AMERICAN DROSOPHILIDAE WITH DESCRIPTIONS
OF TWENTY-THREE NEW SPECIES. Ann. Ent. Soc. Amer. 9:
323-343.
- (35) TRABUT, L.
1923. LE FIGUIER. Bul. Agr. Algerie, Tunisie, Maroc (2) 29:[17]-22,
[33]-39, [49]-60, [73]-75, [97]-101, [117]-124, illus.
- (36) VALLES, F.
1909. IL FICO, NOZIONI BOTANICHE VARIETÀ—COLTIVAZIONE—PRO-
DUZIONE—DISSECCAMENTO—COMMERCIO—AVVERSITÀ. 381 p.,
illus. Catania. (Biblioteca d'agricoltura e industria affini,
v. 26.)

TEST TO DETERMINE TOXICITY OF PYRETHRUM VAPORS TO HONEYBEES¹

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INTRODUCTION

The insecticidal properties of pyrethrum flowers have been known and utilized for more than a century, but neither the chemistry of the toxic principle nor the exact method of toxication is fully understood. In experiments with pyrethrum as a possible substitute for arsenicals in the control of chewing insects it became necessary to ascertain whether or not the volatile material emanating from the flowers or extracts of pyrethrum have deleterious effect upon insects. This question arose as a result of the following experiment. Tent caterpillars (*Malacosoma americana*) transferred to apple trees sprayed 1 hour previously with an alcoholic suspension of pyrethrum flowers died within 24 hours, but when caterpillars were transferred to the same trees 5 days later they fed normally, spun their tent, passed through the third and fourth instars, and continued feeding until practically all the foliage was devoured, with no apparent injurious effects. The apple trees were growing in the greenhouse, and there was no chance for rain or wind to remove the spray material. The question therefore arose whether the cessation of toxicity was due to decomposition of pyrethrum when exposed to light or to exhaustion of the volatile material emanating from it.

REVIEW OF LITERATURE

Among the earlier investigators Hanamann (6)² concludes that the toxicity of pyrethrum is due to its ethereal oils. Dal Sie (2) ascribes the toxic properties to volatile acids. On the other hand, De Bellesme (1) states that the essential oils of pyrethrum are harmless to insects. The work of Hirschsohn (?) indicates that the active principle is nonvolatile, since samples of insect powder kept for several days in paper bags were as active as fresh powder. Similar results were obtained by Gillette (4). Eymard (3) distilled pyrethrum flowers with water. The distillate possessed a strong pyrethrum odor, but proved nontoxic to insects. From the results of more recent investigations carried out by Yamamoto (19), Staudinger and Ruzicka (9, 10, 11, 12, 13, 14, 15, 16, 17, 18), and McDonnell and his associates (8) it seems certain that pyrethrum owes its main toxic properties to a

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² Reference is made by number (italic) to Literature Cited, p. 1056.

mixture of two complex esters, presumably pyrethrin I ($C_{21}H_{30}O_3$) and pyrethrin II ($C_{22}H_{30}O_5$). This, however, does not exclude the possibility that the essential oils of pyrethrum may also possess insecticidal properties.

METHODS

In order to settle this point definitely, the volatile material from finely ground flowers and extracts of pyrethrum was tested on honeybees. Two different methods were employed.

FIRST METHOD

A concentrated alcoholic extract of pyrethrum flowers possessing a strong odor of pyrethrum was placed in Petri dishes or in small beakers covered with screen wire and cheesecloth. A wire cage containing bees and a mixture of honey and water was placed over the Petri dish. The cage was then covered with a large beaker, the inner walls of which were coated with the pyrethrum extract. In this manner the bees were surrounded with pyrethrum vapors. Checks were run in a similar manner, except that the pyrethrum extract was excluded.

SECOND METHOD

Respiration chambers consisting of two large glass dishes (3 inches deep and 7 inches in diameter), made air-tight by heavy rubber bands (3 inches wide), were prepared. (Fig. 1.) A similar apparatus for growing plants under controlled atmospheric conditions has already been described in detail elsewhere (5). Strips of filter paper were

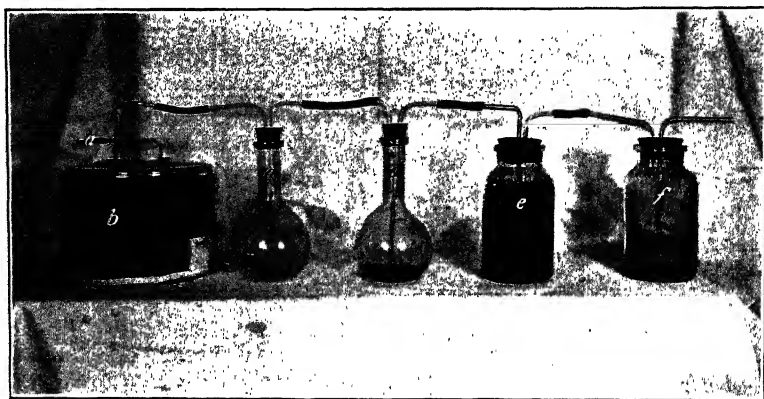


FIGURE 1.—Apparatus used in testing the effect of pyrethrum vapors upon honeybees by the second method: *a*, Tube leading to suction pump; *b*, respiration chamber; *c* and *d*, empty reservoirs to prevent entrance of dust into *b*; *e*, reagent bottle containing pyrethrum extract; *f*, reagent bottle containing ground pyrethrum flowers

placed on the bottom and sides of the dish in order to furnish convenient resting places for the bees. Inside the chamber was placed a watch glass containing cheesecloth saturated with a mixture of honey and water in equal proportions. The upper dish had two holes into which glass tubes were inserted through rubber stoppers. One glass tube was loosely plugged up with nonabsorbent cotton and connected to a series of four reagent bottles, as shown in Figure 1. Bottles *e*

and *f* contained pyrethrum extract and ground flowers, respectively, while bottles *c* and *d* were empty and served as reservoirs to prevent the dust or liquid pyrethrum from reaching the chamber. The second glass tube (*a*) from the upper dish of the chamber (*b*) was connected to a suction pump, by means of which constant aeration was maintained. The glass tubes in the reagent bottles were so arranged that the air bubbled through the pyrethrum extract. The air thus passing through the reagent bottles carried the pyrethrum vapors with it into the chamber.

From 25 to 45 bees were introduced into each chamber and allowed to remain there for 48 hours or longer. Checks were run simultaneously in identically the same arrangement, except that water was substituted for pyrethrum extract and inert powder for ground pyrethrum flowers. Extreme precautions were taken to prevent the particles of pyrethrum flowers or extract from reaching the bees in the chamber. The glass tubes were arranged in such a way that air carrying the volatile material circulated through the entire depth of the chamber before leaving it, thus assuring contact between vapor and insects.

RESULTS

Throughout the experiment the bees receiving pyrethrum vapor did not in any way differ from those in the check chambers. In each case the bees were active, fed on the honey, and appeared normal. The results presented in Table 1 indicate that the insects were not at all affected by the volatile material emanating from pyrethrum. Of the 156 bees kept in the wire cages and respiration chambers containing pyrethrum, 2 died, while of the 161 bees in the check cages and chambers 1 died, mortality being very low in each case.

TABLE 1.—*Effect of pyrethrum vapors on honeybees*

Test No.	Wire-cage method				Respiration-chamber method			
	Pyrethrum		Check		Pyrethrum		Check	
	Bees in cage	Bees dead after 48 hours	Bees in cage	Bees dead after 48 hours	Bees in chamber	Bees dead after 48 hours	Bees in chamber	Bees dead after 48 hours
1.....	26	0	28	0	27	0	24	0
2.....	15	0	32	0	41	1	44	1
3.....					37	1	33	0

The negative results here obtained can be explained in two ways: (1) The essential oils of pyrethrum exert no toxic effect on insects; (2) the rate of volatility may be too low to produce a lethal concentration in the atmosphere. In either case the insecticidal properties of pyrethrum vapors should be considered of no importance from the practical standpoint. If the air in the closed chamber, which was practically saturated with pyrethrum vapors (because it passed through flowers and bubbled through liquid extract of pyrethrum), did not contain a lethal dose, the possibility that the atmosphere in the open, surrounding the sprayed foliage, should contain a lethal concentration is rather remote. The cessation of toxicity of the

alcoholic pyrethrum suspension to tent caterpillars after five days' exposure on apple foliage was therefore not due to the exhaustion of the essential oils but to other factors, which possibly decomposed the active principle.

SUMMARY AND CONCLUSION

Honeybees were kept in chambers through which pyrethrum vapors were continuously circulated. At the end of 48 hours the bees appeared normal and were evidently not affected by the volatile material emanating from pyrethrum.

It appears, therefore, that the toxicity of ground flowers, as well as extracts of pyrethrum, is primarily due to the nonvolatile substances present, namely, pyrethrine I and pyrethrine II, while the essential oils do not in any way affect the insects.

LITERATURE CITED

- (1) BELLESME, J. DE.
1876. SUR UN ALCALOÏDE DU PYRETHRUM CARNEUM. Jour. Pharm. et Chim. (4) 24: 139.
- (2) DAL SIE, G.
1879. SUR LE PRINCIPE ACTIF DE LA POUDRE INSECTICIDE. Bul. Soc. Chim. France (n. s.) 31: 542-543.
- (3) EYMARD, L.
1890. RECHERCHES SUR LA POUDRE DE PYRÈTHRE DU CAUCASE. Union Pharm. 31: 357-361.
- (4) GILLETTE, C. P.
1889. IMPORTANT INJURIOUS INSECTS. PREPARATION OF INSECTICIDES: EXPERIMENTS WITH PYRETHRUM. Iowa Agr. Expt. Sta. Bul. 5: [161]-196, illus.
- (5) GINSBURG, J. M.
1925. A MODIFIED RESPIRATION APPARATUS FOR PLANT AND SOIL STUDIES. Soil Sci. 19: 411-415, illus.
- (6) HANAMANN, J.
1863. ÜBER DAS PERSISCHE INSECTENPULVER. Vrtlj. Prakt. Pharm. (Wittstein) 12: 522-525.
- (7) HIRSCHSOHN, E.
1890. BEOBACHTUNGEN ÜBER DEN WIRKSAMEN BESTANDTHEIL DES INSECTENPULVERS. Pharm. Russland 29: 209-213.
- (8) McDONNELL, C. C., ROARK, R. C., LA FORGE, F. B., and KEENAN, G. L.
1926. INSECT POWDER. U. S. Dept. Agr. Bul. 824, 95 p., illus.
- (9) STAUDINGER, H., MUNTWYLER, O., RUZICKA, L., and SEIBT, S.
1924. INSEKTENTÖTENDE STOFFE VII. SYNTHESSEN DER CHRYSANTHEMUM-SÄURE UND ANDEREN TRIMETHYLEN-CARBONSÄUREN MIT UNGESÄTTIGTER SEITENKETTE. Helvetica Chim. Acta 7: 390-406.
- (10) ——— and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE I. ÜBER ISOLIERUNG UND KONSTITUTION DES WIRKSAMEN THEILES DES DALMATINISCHEN INSEKTENPULVERS. Helvetica Chim. Acta 7: 177-201.
- (11) ——— and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE II. ZUR KONSTITUTION DER CHRYSANTHEMUM-MONOCARBONSÄURE UND -DICARBONSÄURE. Helvetica Chim. Acta 7: 201-211.
- (12) ——— and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE III. KONSTITUTION DES PYRETHROLONS. Helvetica Chim. Acta 7: 212-235.
- (13) ——— and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE IV. KONSTITUTION DES TETRAHYDRO-PYRETHRONS. Helvetica Chim. Acta 7: 236-244.
- (14) ——— and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE V. SYNTHESE DES TETRAHYDRO-PYRETHRONS, DES REDUKTIONSPRODUKTES DES PYRETHROLONS. Helvetica Chim. Acta 7: 245-259.

-
- (15) STAUDINGER, H., and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE VI. UNTERSUCHUNGEN ÜBER CYCLOPENTANOLONDERIVATE UND IHR VERGLEICH MIT DEM PYRETHROLON. *Helvetica Chim. Acta* 7: 377-390.
- (16) ——— and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE VIII. VERSUCHE ZUR HERSTELLUNG VON PYRETHROLONÄHNLICHEN ALKOHOLEN. *Helvetica Chim. Acta* 7: 406-441.
- (17) ——— and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE IX. WEITERE VERSUCHE ZUR HERSTELLUNG VON CYCLOPENTANOLONDERIVATEN MIT UNGESÄTTIGTER SEITENKETTE. *Helvetica Chim. Acta* 7: 442-448.
- (18) ——— and RUZICKA, L.
1924. INSEKTENTÖTENDE STOFFE X. ÜBER DIE SYNTHESE VON PYRETHRINEN. *Helvetica Chim. Acta* 7: 448-458.
- (19) YAMAMOTO, R.
1919. THE INSECTICIDAL PRINCIPLE IN CHRYSANTHEMUM CINERARIAEFOLIUM. I. *Jour. Tokyo Chem. Soc.* 40: 126-147. [(Abstract) *Chem. Abs.* 13: 1221.]



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No. 12

STUDIES OF THE STAMINATE INFLORESCENCE AND POLLEN OF *HICORIA PECAN*¹

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INTRODUCTION

From the producer's standpoint one of the most important limiting factors to profits in pecan culture is low yields. These may be due to any one or to a combination of several factors, such as the failure of the trees to differentiate flowers, more especially pistillate flowers, poor pollen, unfavorable weather during the flowering season, or insect or disease attack upon flowers or developing fruits. Considerable study has been devoted to some of these questions. Little, however, has been recorded regarding the development of pollen in the pecan, though pollen deficiencies are known to be responsible for unsatisfactory fruit setting in certain varieties of apples, pears, plums, grapes, and many other fruits. It therefore appeared to the writer that a study of the morphology of the staminate inflorescence and physiology of the pollen would throw some light on practical questions of pollination.

REVIEW OF LITERATURE

Unfruitfulness of the pecan (*Hicoria pecan* Brit.) is associated more closely with the initiation and development of pistillate than of staminate flowers, and it is therefore logical that the pistillate flowers should have been studied first. Studies of the morphology of pistillate flowers of pecans have been made by Woodroof (27, 28),³ Shuhart (21), and Isbell (12), and the results have shown close agreement as to the time and manner of development. Staminate flowers have received secondary attention, but fragmentary descriptions have appeared in a few reports (23, 26, 12, 27, 28).

Stuckey (23) studied the development of staminate flowers of 33 varieties of pecans. He divided the varieties into two groups on the basis of the length and size of the catkins, length of bracts, and time of shedding pollen. Woodroof (26, 27) found that catkins were differentiated in lateral buds throughout the growing season of

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² In the investigations here reported Naomi C. Woodroof, of the Department of Botany, and J. E. Bailey, of the Department of Horticulture, of the Georgia Agricultural Experiment Station, aided in collecting field data and in preparing material for laboratory studies. Otis Woodard, of the Georgia Coastal Plain Experiment Station, furnished a part of the data for Table 5; C. F. Williams, of the North Carolina Agricultural Experiment Station, and R. M. Middleton, of the Georgia station, furnished a part of the data for the same table. Members of the Department of Horticulture of Michigan State College offered suggestions in the planning of the work and criticized the manuscript; and W. C. Dutton aided in making photomicrographs.

³ Reference is made by number (italic) to "Literature cited," p. 1103.

the year prior to the shedding of pollen. He reported measurements of catkins of the two groups of varieties during the winter stage and described the method of shedding bud scales the following spring. Isbell (12) published 13 photomicrographs illustrating progressive stages of catkin development during the summer.

Higgins (10) described a disease (*Microstroma juglandis* (Bereng.) Sacc., var. *robustum*, n. var.) of pecan catkins that appeared on all of the varieties observed and sometimes destroyed one-third of the pollen. The tissues of the catkin were not killed outright, but the pollen grains degenerated, often leaving collapsed walls. This disease is common in the orchard in which the present studies were made, but care was taken to avoid using diseased material.

Schuster (18) stated that the amount of pollen produced by filberts in Oregon varies from season to season and is influenced by many factors. Many varieties produce a heavy crop of staminate and pistillate flowers one year, and a light crop of both the following year.

Valleau (24), working with strawberries, Dorsey (8) with plums, Knowlton (15) with the J. H. Hale peach, and Asami (3) with Shanghai peach, report that the first abnormal development in pollen was seen at the time of liberation from the tetrad wall; thereafter microspores were found in various stages of abortion. Shoemaker (19, 20) found lagging chromosomes in the heterotypic division, giving rise to abnormal pollen in the apple and cherry.

The germination of pollen on artificial media has been unsatisfactorily accomplished with pollen of many plants. Wood⁴ found that almond and walnut pollen did not germinate well on sugar-agar media, and concluded that there was no satisfactory laboratory method of ascertaining the viability of pollen of various varieties. Anthony and Harlan (2) attempted to duplicate natural conditions for germinating barley pollen by placing a piece of mesophyll from the leaf of the garden pea in the cell to supply water. The cell was covered with a cover glass and placed in the open to allow the condensation of moisture on the pollen grains. Germination was accomplished with both mesophyll and drops of water for humidifiers. Beaumont and Knight (5) and Knowlton (15) attempted to approach natural conditions for germinating apple pollen by adding stigmas of the same or different varieties to a hanging drop at the same time the pollen was added. They concluded that further improvement of the method of germination was necessary to produce pollen-tube length equal to that produced under natural conditions. Lidforss (17) reported that artificial media for germinating pollen must contain not only the essential nutritive substances but that it must not contain substances which prevent growth, especially mineral salts, as calcium.

The most satisfactory results, from pollen-germination tests in general, have been obtained by using from 1 to 2 per cent agar or gelatin and 5 to 20 per cent sucrose in the media. Howlett (11) used 10 per cent sucrose and 2 per cent agar; Beaumont and Knight (5) used 5 per cent sucrose and 1 per cent gelatin for germinating apple pollen; Booth (6) used 15 per cent sucrose and 1½ per cent gelatin for germinating plum pollen and 10 per cent sucrose and 5 per cent gelatin for germinating cherry pollen; Schuster (18) obtained best germination of filbert pollen in 12 to 15 per cent sucrose but observed bursting of

⁴WOOD, M. N. POLLEN STUDIES OF ALMOND AND WALNUT. 1917. (Master's thesis. University of California.)

pollen grains and tubes; Auchter (4) germinated apple pollen in distilled water; and Knight (14) increased apple-pollen germination by adding a trace of asparagine to the medium.

Valleau (24) determined the percentage of defective grains in strawberry pollen and reported that—

lactic acid has the advantage over water or alcohol for this purpose as it is not volatile and seldom, if ever, breaks the pollen grains through osmotic pressure. It readily enters and expands the normal grains, while it leaves the aborted grains collapsed.

Dorsey (8) studied plum pollen and reported that the extent of pollen abortion in selected forms was determined partly from mounts in lactic acid and partly from stained sections. In Shoemaker's (19) work with apples lactic acid was employed to afford a liquid medium which would prevent bursting and germination of the pollen grains. Beaumont and Knight (5) hand-pollinated apple blossoms, and after the pollen had germinated and penetrated the stigmas the latter were flattened on a cover glass and mounted in lactic acid which killed and fixed the pollen tube. Snow (22) used lactic acid in a similar manner in studying the pollen of stocks. Florin's (9) germination tests closely checked with the lactic acid examinations of apple and pear pollen.

METHODS

Material for morphological studies was taken from an orchard of 28 varieties at the Georgia Experiment Station. The location is about 150 miles north of the center of pecan production in Georgia. Most of the trees are 20 years old and have had uniform culture. Data on pollen dissemination were taken in commercial orchards near Barnesville, Ga.

In general, the methods of collecting, killing, fixing, embedding, sectioning, and staining material for microscopical studies were the same as those described in previous reports (27, 28, 31).

The Alley and Frotscher varieties were chosen as typical of Groups 1 and 2, respectively, according to Stuckey's classification (23). Other varieties were repeatedly used to verify results. Juel's fixative⁵ and picro-sulphurous acid⁶ were used as killing and fixing agents, the latter giving the better results. Material was taken at 15-day intervals throughout the year from each variety, alternating the fixatives. Normal buds were selected from representative shoots, and normal flowers were taken from catkins typical of the variety. From the mother-cell stage until pollen shedding, catkins of the Stuart variety were collected daily; and during the reduction division, material of the Frotscher and Stuart varieties was collected at 3-hour intervals. Buds collected during the actively growing season were sectioned 5 microns in thickness. The hairy nature of winter buds rendered it difficult to section them sufficiently thin for cellular studies.

A modification of the smear method, as described by Kaufmann (13), was used to determine the number of tetrads per anther and the number of pollen grains per pollen sac. An anther in the tetrad stage was placed in a drop of water on a slide and thoroughly macerated with a needle. The tetrads separated from the anther wall and floated independently in the medium. After the fragments of the anther

⁵ Juel's fixative: ZnCl_2 , 2 gm.; acetic acid, 2 c. c.; alcohol, 95 per cent, 50 c. c.; distilled H_2O , 50 c. c.

⁶ Picro-sulphurous acid: Sulphurous acid, 6 per cent, 10 c. c.; picric acid, $1\frac{1}{2}$ gm.; alcohol, 75 per cent, 90 c. c.

wall were removed the tetrads were stained with eosin, covered with a cover glass, and examined under the high power of the microscope.

Dissemination of pollen was studied by catching pollen from the air on slides greased with vaseline, a method similar to that used by Waugh (25) for catching plum, pear, and apple pollen. Two yardsticks wrapped with cheesecloth and loosely bolted together made a convenient holder for 12 slides. About two-thirds of the length of each slide was exposed, the remainder being held firmly between the yardsticks. Three series of slides were mounted on a slender pole,

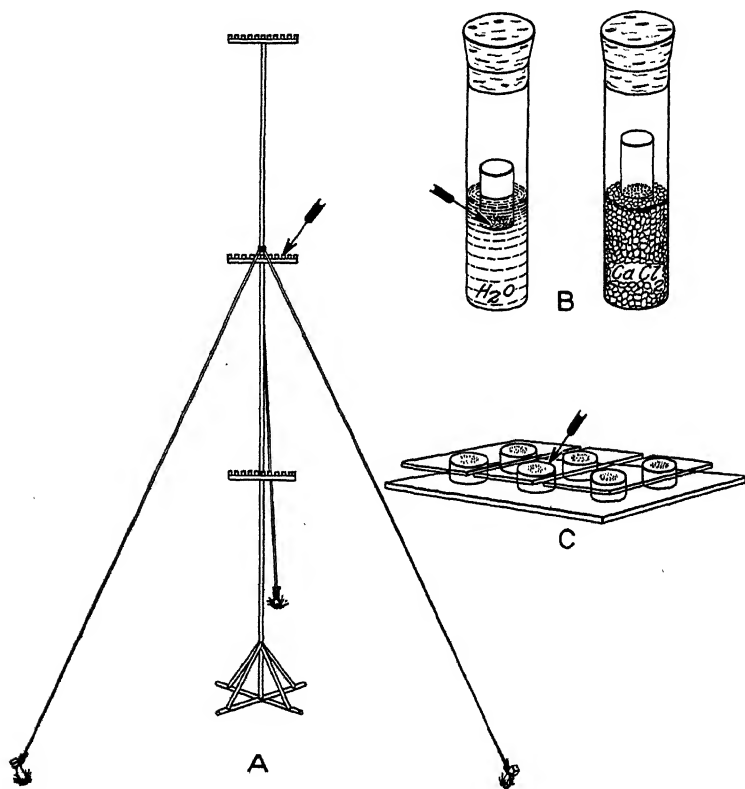


FIGURE 1.—A, Pole 30 feet high supported by guy wires to which were attached three series of greased slides for catching pollen from the air; B, vials containing water and calcium chloride, in which were placed smaller vials containing pecan pollen which was kept "moist" and "dry" respectively; C, cells for germinating pollen. Arrows in each case indicate position of the pollen.

one series each 10, 20, and 30 feet above the ground. The slides were set on the leeward side of the trees, at a 45° angle with the ground, the greased face of the slide being turned toward the pollen-shedding trees. The slides were changed by lowering and raising the pole with three guy wires. (Fig. 1, A.)

Pecan pollen grains on the greased slides were identified and counted under the low-power objective of the microscope. After the area of the microscopical field was calculated, the number of grains in 200 fields were counted, averaged, and calculated to the number of grains per square centimeter.

The wind velocity was determined by the use of a Robinson cup anemometer with electric connections and a buzzer which indicated the number of miles per hour. The relative humidity was determined by the use of a stationary hygrometer placed in the orchard.



FIGURE 2.—A, Microspore print made from a pecan catkin of Group 1 showing the ease with which pecan pollen may be collected in quantity; B, photomicrograph of longitudinal section of Alley variety anther while in the mother-cell stage on April 1; in some cases there seem to be rows of mother cells

Pollen for germination tests was obtained from ripe anthers which were allowed to dehisce in the laboratory. (Fig. 2.) In this way the time of dehiscence could be determined to within two hours. Cells for germinating pollen were made by shellacking six hard

rubber rings, 15 mm. in diameter and 10 mm. high, to glass slides 2 inches wide and 3 inches long. Water to supply moisture for germination was placed in the bottom of each cell. A drop of sterile germinating medium was placed on a cover glass while hot and allowed to solidify. The pollen was dusted on the medium and the cover glass was inverted and sealed on the cell with vaseline. Examinations were made at intervals under low power without disturbing the cell. (Fig. 1, C.)

Dry and moist storage conditions were provided by placing calcium chloride and water, respectively, in two 50 c. c. vials. A 10 c. c. vial containing pollen was placed in each 50 c. c. vial and the latter was corked. (Fig. 1, B.) A duplicate series was set up for each variety and temperature. Electric ovens provided tempera-

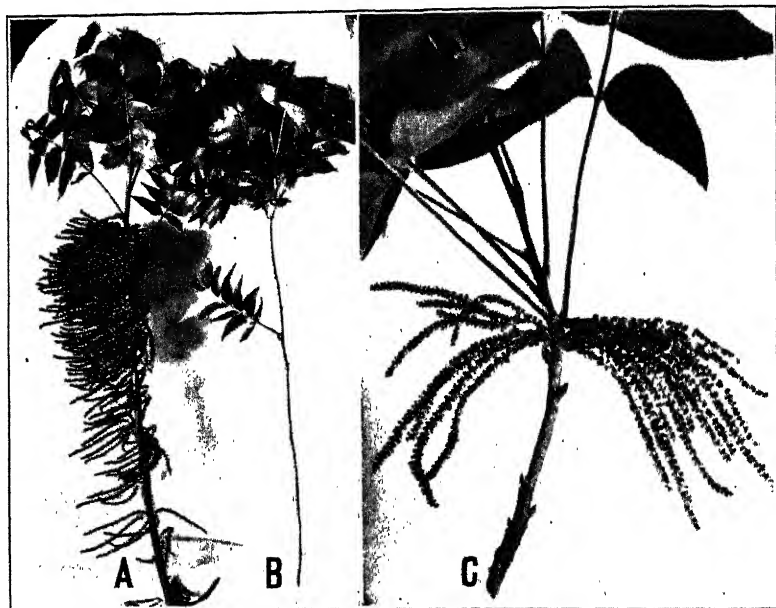


FIGURE 3.—A, Twig from Stuart pecan tree which bore 3 pounds of nuts last year; B, twig from Mobile tree which bore 29 pounds of nuts last year. This tree and that in A are both 12 years old and have received the same fertilizer and cultural treatments since being set in the orchard. C, Twig from McAllister variety, a supposed hybrid between *Ilcioria pecan* and *Carya alba*, which has a catkin producing habit like *C. alba*, i. e., only the terminal bud produces catkins

tures constant at 32°, 25°, 23°, and 22° C.; and a refrigerator provided a temperature of 5° C.

Mounting pollen in lactic acid for microscopical determination of the percentage of defective grains seems a more reliable method than germinating it on artificial media. Two drops of 90 per cent lactic acid placed on a slide with a small amount of pollen and covered with a cover glass render the grains containing a normal amount of protoplasm distinct from those containing less than the normal amount. About three minutes are required for complete penetration; after three hours the grains become bleached and differentiation of defective and normal grains is uncertain. Fresh mounts and high magnification are necessary for one to make reliable counts by this method.

PRESENTATION OF EXPERIMENTAL DATA

The lateral buds of a growing shoot on a bearing pecan tree differentiate catkin primordia by the time the subtending leaf is one-tenth grown (26, 27). Differentiation begins about two weeks after active growth starts in the spring (April 15) and continues throughout the growing season. In the pecan, catkins are always produced in lateral, axillary buds (figs. 3, 4), and the number of catkin primordia formed is in proportion to the number of leaves produced on that shoot. Normally there are two groups of three catkins each produced at each node. In other native hickories (*Carya alba*, *C. ovalis*, *C. glabra*) the catkins are produced in the terminal bud. (Fig. 3, C.)

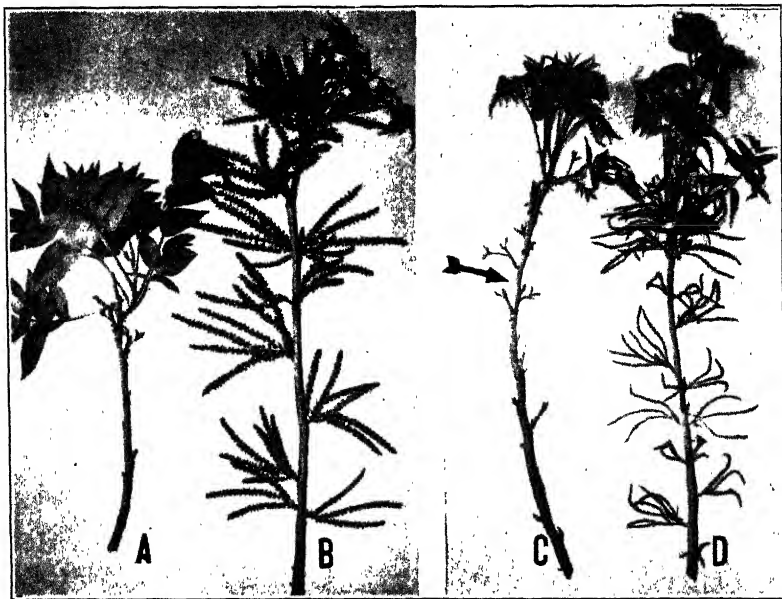


FIGURE 4.—A, Twig from Mobile tree which bore 58 pounds of nuts last year; tree from variety of Group 1; B, twig from Alley tree which bore 34 pounds of nuts last year; tree from variety of Group 1; C, twig from Frotscher variety which bore 100 pounds of nuts last year; tree from variety of Group 2; D, twig from Stuart tree which bore no nuts last year; tree from variety of Group 2. All of these trees are 20 years old and have received the same fertilizer and cultural treatments since being set. Arrow indicates point of abscission of the catkin buds on April 9

The characteristics of the buds and inclosed catkin primordia have been previously described by the writer (26). The central catkin is formed and develops somewhat in advance of the catkins borne on either side, and ultimately reaches a greater length at maturity. (Figs. 5, 6, 4.)

THE TWO GROUPS OF VARIETIES

Stuckey's (23) description of the two groups of varieties was based on characteristics of the catkins at the time of pollen shedding. The writer (26) found that the catkin primordia of Group 1 have a greater diameter and lesser length than those of Group 2 in both October and January (26). Such measurements aid in differentiating the

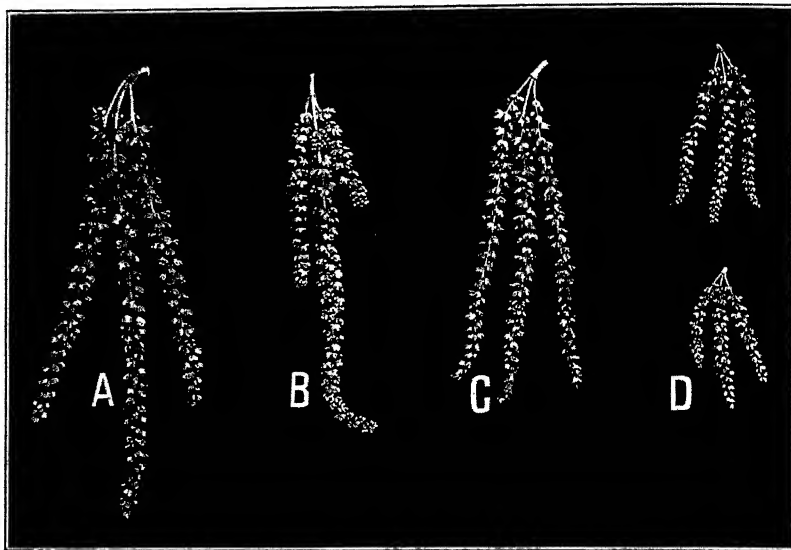


FIGURE 5.—Catkins of pecan varieties in Group 1: A, Alley; B, Jerome; C, Mobile; D, Mobile from nonvigorous bud (0.57 natural size)

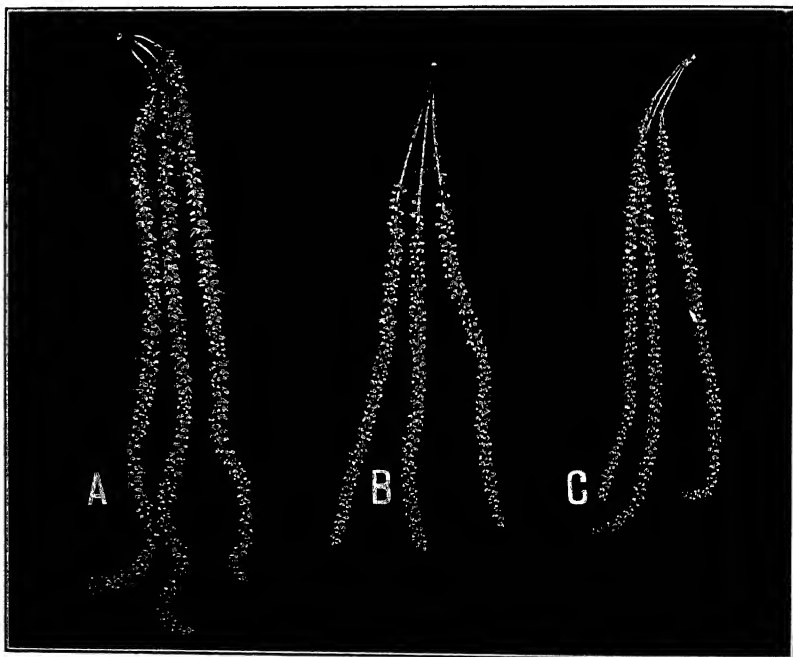


FIGURE 6.—Catkins of pecan varieties in Group 2: A, Van Deman; B, Frotscher; C, Teche. The Frotscher and Teche varieties have a large number of aborted flowers near the base of the cluster (0.57 natural size)

two groups, but as diameter and length vary with the vigor of the tree, they can not be depended upon solely.

There seems to be no difference between the mode of differentiation and type of growth of the catkins of the two groups of varieties during the spring and summer, but a marked difference is evident by early fall.

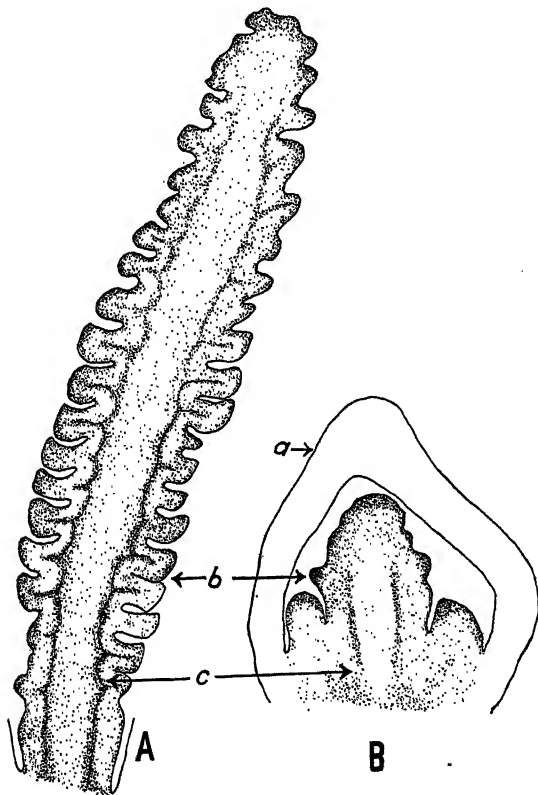


FIGURE 7.—Primordia of staminate inflorescence: B, Initial formation of a group of three catkins within a single bud scale, May 15; A, single inflorescence six weeks later: a, bud scale; b, primordium of an individual flower; c, initial formation of vascular bundles. Protscher variety. $\times 131$

Individual flower primordia develop as lateral "bumps" on the axis of the catkin primordium, beginning at the base and progressing toward the terminus. (Fig. 7.) In Group 1 flower primordia continue to form at the terminus, while the older ones near the base differentiate anthers by early fall. By the time growth practically ceases in the fall anthers are distinguishable along the entire length of the axis. (Figs. 8 and 9 and Table 1.) Thus the winter stage of Group 1 shows complete differentiation of anthers and bracts.

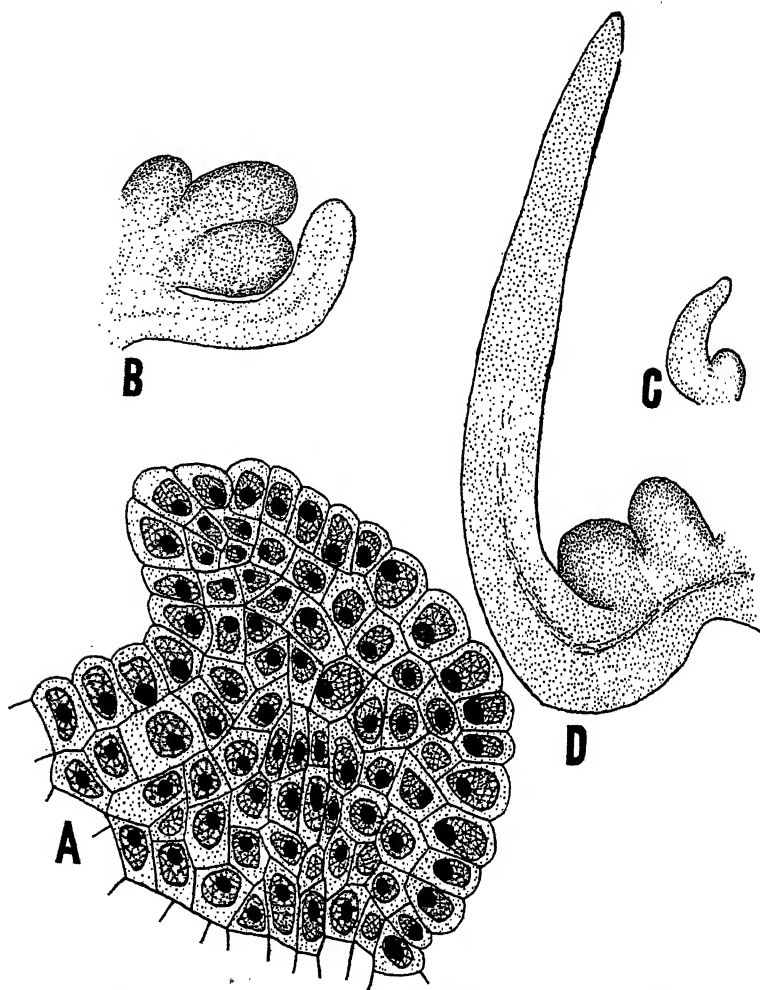


FIGURE 8.—A, Individual flower primordium showing specialization of cells but no differentiation of floral organs; enlargement of Figure 7, $\times 1040$; B, Alley variety on March 15 showing floral organs well differentiated; $\times 114$; C, Frotscher variety on March 15 showing very early stage in differentiation of floral organs; $\times 114$; D, Frotscher variety on April 1 showing floral organs in same stage as Alley on March 15; $\times 114$

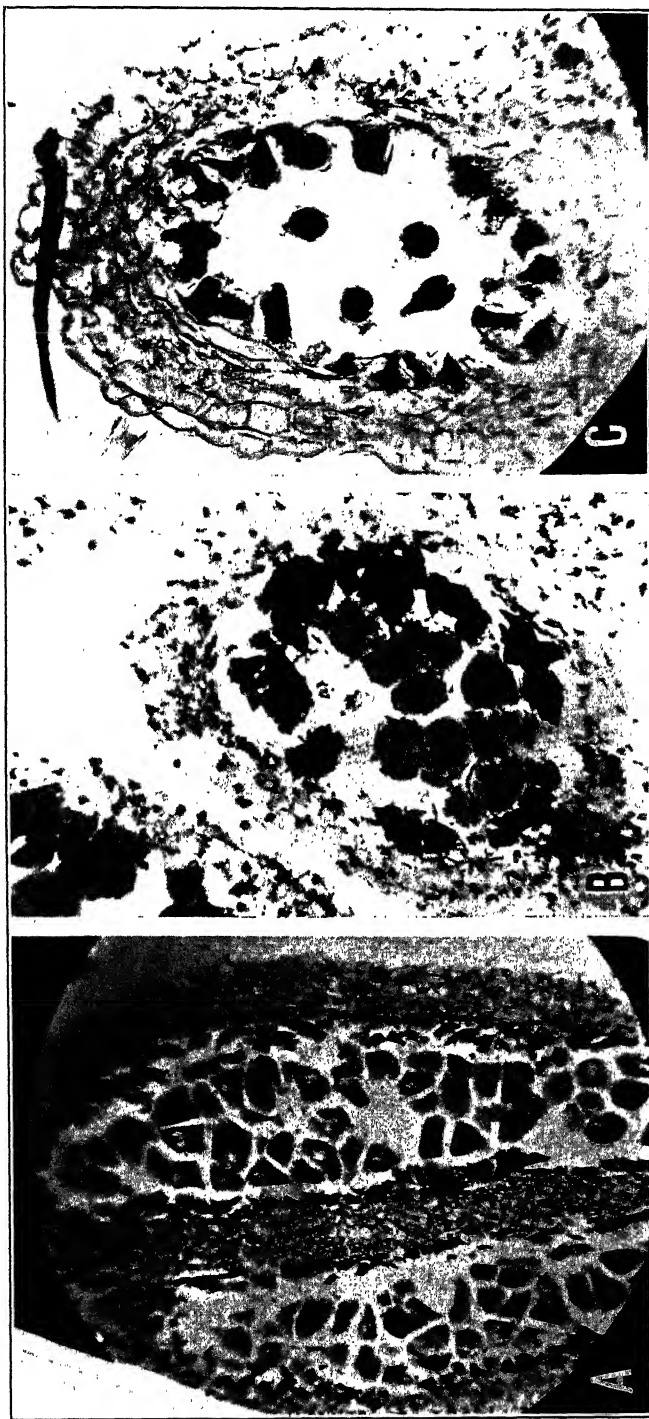


FIGURE 9.—A, Photomicrograph of longitudinal section of two lobes of an anther showing mother cells just before rounding up; B, photomicrograph cross section of one lobe of an anther showing mother cells disintegrating, and tetrads within the mother cell walls; one tetrad is elongated, indicating abnormality

TABLE 1.—*Stages of development of pollen of pecan varieties in Groups 1 and 2*

Stage of development	Groups at stage of development mentioned at time indicated															
	April	May	June	July	August	September	October	February	March			April			May	
									1	10	20	1	10	20	1	10
Differentiation of inflorescence.....	1, 2	1, 2	1, 2	1, 2	1, 2	1, 2										
Differentiation of anthers.....						1		1	2	2	2					
Formation of anther lobes.....											1	1, 2	2			
Archisporial cells.....											1	1, 2	2			
Mother cells.....												1	1, 2	2		
Rounding-up of mother cells.....												1	1, 2	2		
Reduction division.....												1	1, 2	2		
Tetrads.....													1	1, 2	2	
Microspore liberation.....														1	1, 2	
Anther dehiscence.....															1	2

In Group 2 the flower primordia continue to form as long as growth continues in the fall, but no differentiation of anthers occurs

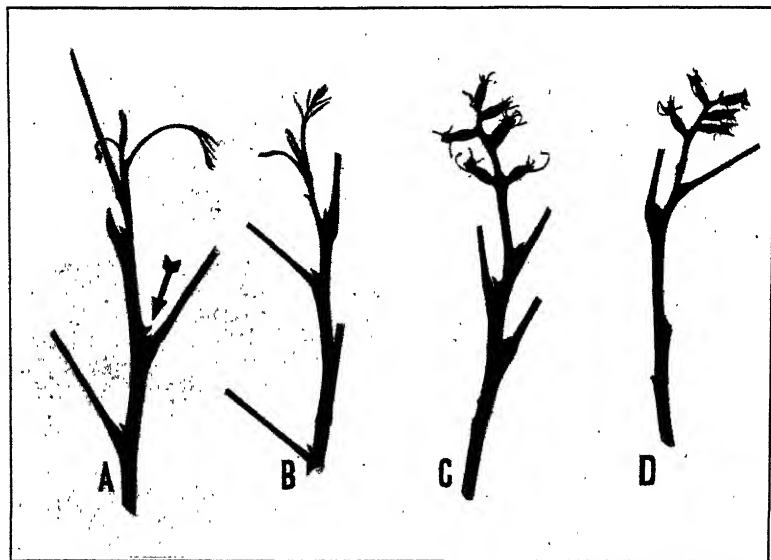


FIGURE 10.—Unfruitful (A, B) and fruitful (C, D) twigs; the auxiliary buds on both types of shoots produce staminate flowers the following spring (0.48 natural size)

until the following spring. Thus the individual flower primordia reach an inactive stage soon after formation and remain so for from four to nine months, while additional flower primordia continue formation toward the actively growing terminus. (Fig. 7.) Thus catkins of varieties of Group 2 are longer than those of Group 1. The period of inactivity varies with the time of formation—the basal and first-formed ones remain inactive longer than the newly formed terminal ones; also those formed in buds near the base of the new shoot early in the season are inactive for a longer time than those toward the terminus of the shoot. (Fig. 10.)

From early fall until the anthers are mature the following spring the development of the catkin primordia and the catkins of Group 1 is considerably in advance of that of Group 2. In the fall the difference is from 2 to 3 weeks; in winter when growth is almost at a standstill the maximum difference of 3 or 4 months occurs; while in spring when growth is most rapid the difference is from 10 to 15 days (figs. 4, 5, 6, and 11), and under some seasonal conditions the shedding of pollen of the two groups may be nearly coincident. (Table 5.)

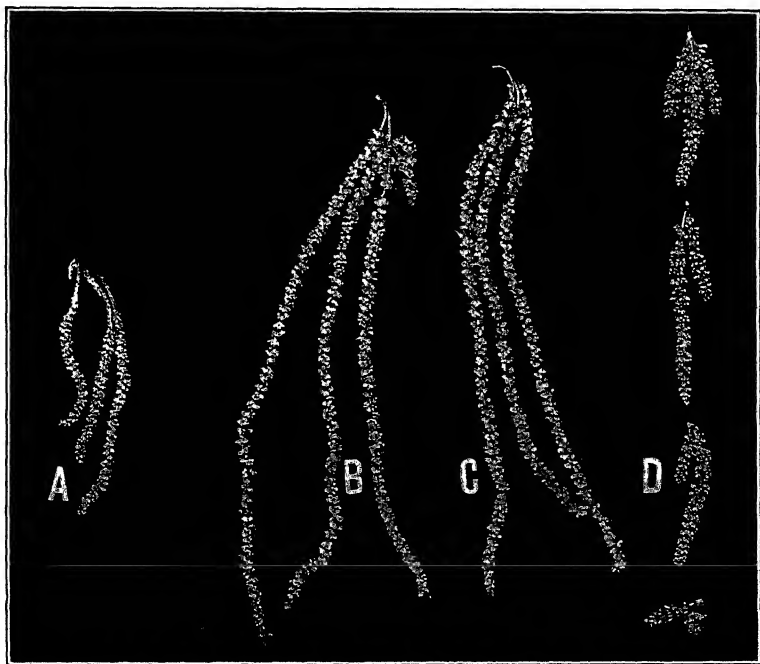


FIGURE 11.—A, Weak catkins with aborted flowers at base; B, vigorous catkins branched at base; C, normal catkin; D, aborted flowers from weak shoots. Indiana variety. (0.55 natural size)

The varieties of pecans can be separated into two groups in winter on the basis of the internal appearance of the lateral buds. Carefully cut and stained free-hand sections are sufficient to enable one to make the distinction. Practical use has been made of this fact in determining in advance the approximate date on which pollen of a new or unknown variety should shed.

The practical significance of the time of pollen shedding of the two groups will be discussed later. Anthers of varieties of Group 1 are differentiated several months before pollen shedding and differentiation occurs during the previous growing season, while differentiation of anthers of varieties of Group 2 occurs about two months before pollen shedding and takes place during the current growing season, as is the case with pistillate flowers (28, 21, 12). A closer relation thus exists in respect to time of differentiation between staminate and pistillate flowers of Group 2 than between staminate and pistillate flowers of Group 1.

INFLORESCENCE

A catkin primordium consists of three regions.

(1) The interior region occupies, as seen in longitudinal section, almost one-half of the diameter of the primordium. It consists entirely of large, slightly elongated, parenchyma cells, extending longitudinally with the axis. These remain parenchyma cells throughout the life of the catkin and are analogous to pith cells in a young shoot. The diameter of the spherical nuclei is about one-third the smallest diameter of the cell. The nuclei and cytoplasm do not stain as heavily as in the parenchyma cells of the primordia of individual flowers.

(2) Surrounding the interior region is a cylinder of small, narrow cells, with elongated nuclei which more than half fill the cells. The outline of this region is slightly irregular in both cross and longitudinal sections. In longitudinal section the cells vary greatly in size and shape and lie very close together. As growth continues these cells become sclerenchyma cells of the vascular bundles which connect each flower with the vascular system of the catkin.

(3) The third region of the catkin primordium consists of individual flower primordia which are made up entirely of parenchyma cells from the time of differentiation until September. (Fig. 8, A.)

In longitudinal section the epidermal cells of the flower primordium are rounded, uniform in size, but somewhat elongated. They lie close to one another and form a complete layer one cell thick over the surface. Directly beneath the epidermal layer are closely appressed 3-sided to 6-sided meristematic cells which vary widely in size. Sections made from material collected August 1 show that some of these cells are several times larger than others and are in a state of division. In the center of a flower primordium are several layers of elongated cells with oblong nuclei. These become sclerenchyma cells of a vascular system connecting each bract and anther with the vascular system of the catkin.

Bracts are differentiated about 10 days before the anthers. The first appearance of the bracts is shown by a turning of the terminus of the flower primordia toward the terminus of the catkin primordium, forming an angle of about 45° . As growth continues the bend becomes less abrupt and a protrusion appears in the axil. This protrusion divides immediately into four or five smaller ones, and each of these becomes an anther. (Figs. 8, 7.)

The development of bracts in spring is very rapid. Three branches soon form, the middle one being broader and longer than those on either side. The three almost envelop the developing anthers. (Figs. 6, 12.) The bracts contain chlorophyll, have numerous hairs on their surfaces and small veins, and presumably function as true leaves.

ANTHER

The anthers of the Frotscher variety on March 5 (40 days before shedding pollen) were a homogeneous mass of parenchyma cells covered by an epidermis. (Fig. 13, B.) Very early the anther appeared 4-lobed in cross section (fig. 14, B); and the differentiation of the vascular strand of the connective tissue outlined the general plan of structure. Almost simultaneously with the formation of lobes, a plate of hypodermal (archesporial) cells became differentiated in each lobe,

faintly distinguished from the adjacent cells by their large size, numerous sides, large nucleii, and less dense staining.

Subsequent divisions up to the mother-cell stage follow one another very rapidly. The archesporial cells divide by periclinal walls, form-

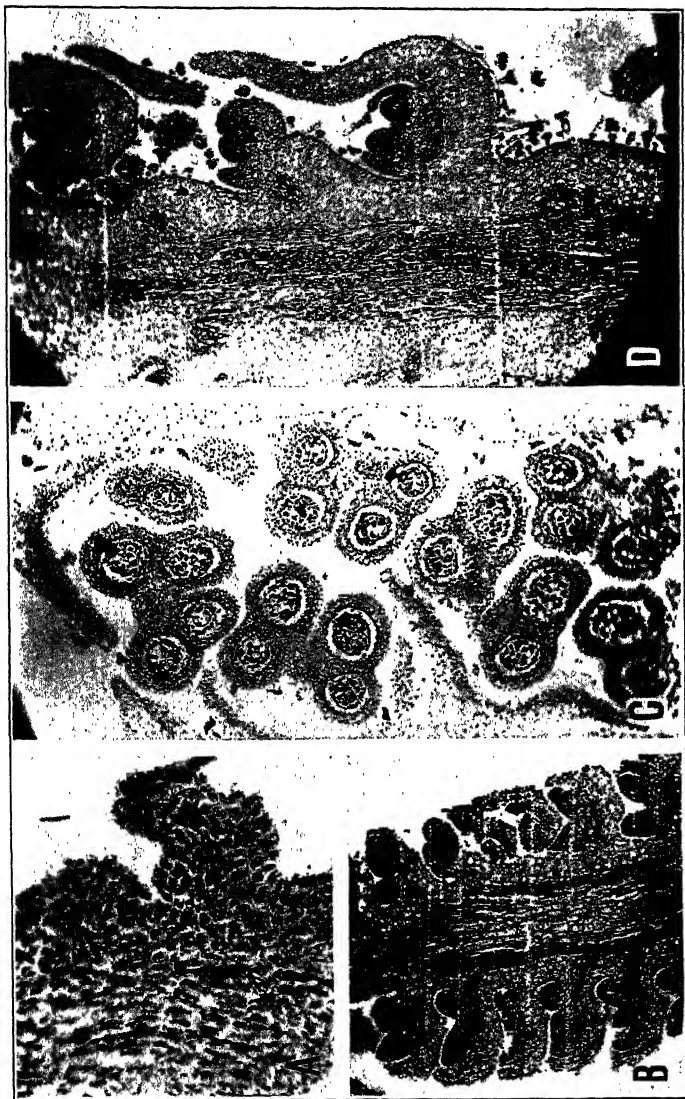


FIGURE 12.—Photomicrographs showing condition of flowers of Groups 1 and 2 on two different dates: A, Individual flower primordium just before differentiation of anthers, Group 2, Frotscher variety, December 1; B, longitudinal section of a catkin showing anthers formed in the Alley variety on December 1 as is typical of varieties in Group 1; C, cross section of several anthers showing tapetum cells broken down and mother cells separating, Alley variety, Group 1, April 1; D, longitudinal section of flowers of Frotscher variety, Group 2, on April 1, showing the anthers not yet differentiated into lobes

ing an outer layer of primary parietal (primary tapetum) cells, and an inner layer of primary sporogenous cells; the former producing the wall of the embedded sporangium and the latter the sporogenous tissue. The primary tapetum cells successively divide by periclinal walls until six or seven layers are formed. The inner layer is known

as the endothecium or tapetum, the outer layer as the exothecium or epidermis, the three or four remaining layers as "middle layers." The exothecium cells are thin walled, large and cubical, with large vacuoles and small oval nuclei which adhere to the sides of the cells toward the center of the anther. The cells of the middle layer are much smaller, somewhat compressed, many-sided, thin-walled, contain no vacuoles, have nuclei centrally located, are elongated, and

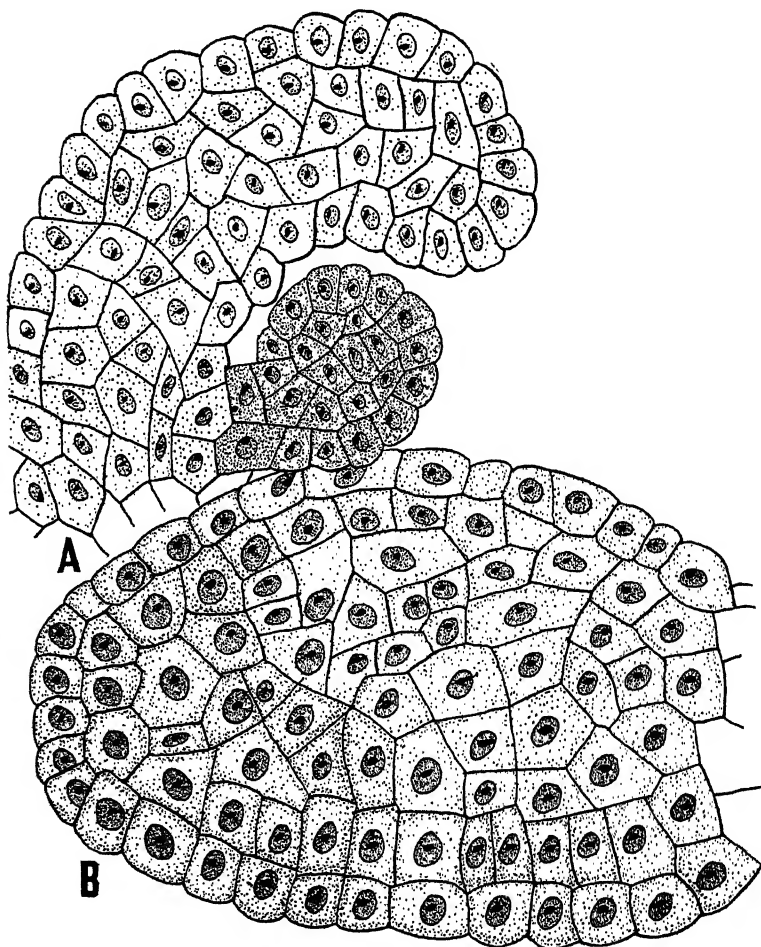


FIGURE 13.—Enlargements of Figure 8: A, Figure 8, $C \times 1,000$; B, Figure 8, $B \times 1,000$

stain lightly. The thick-walled, deeply staining, 6-sided, closely pressed, small nucleate tapetum cells form a jacket about the sporogenous cells, one or two cells in thickness.

Simultaneously with the division of the primary tapetum cells the primary sporogenous cells multiply without definite order to form mother cells. Division apparently occurs in every direction with occasional formation of layers but no definite massulae. Longitudi-

nally, the mass of closely compressed, many-sided, large nucleate mother cells extends almost the length of the anther, and about three cells wide. (Fig. 15, B.) They stain about as deeply as the middle layers but much lighter than the surrounding tapetum cells.

Further development is accompanied by separation, disorganization and disintegration of tapetum cells; and separation, increase in

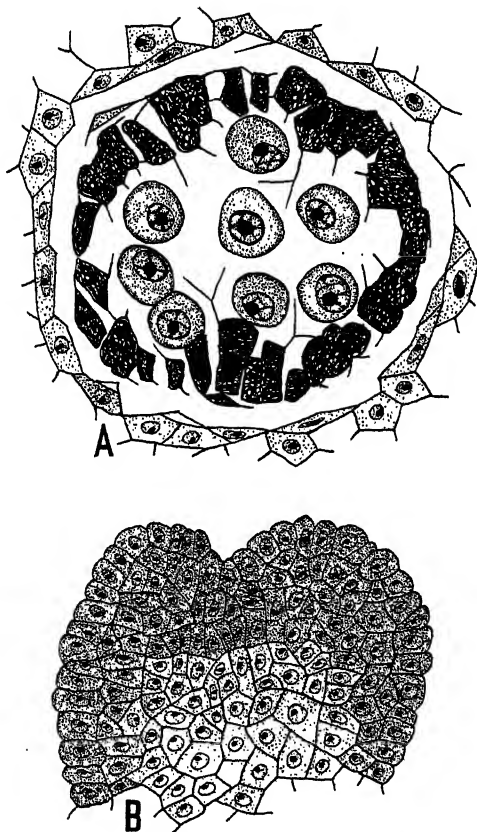


FIGURE 14.—A, Camera lucida drawing of cross section of an anther of Alley variety on April 5, showing rounding-up stage of the mother cells and continued disintegration of the tapetum cells; $\times 410$; B, cross section of an anther of Frotscher variety on April 5, showing lack of differentiation of the interior into typical tapetum and mother cells; $\times 410$

size, increase in thickness of walls, and rounding-up of the mother cells. (Figs. 14 and 16.)

The first evidence of disintegration of the tapetum cells occurs about 35 days before pollen shedding. (Fig. 15, B.) The layer of cells adjacent to the mother cells undergoes nuclear division once or twice, withdraws from the mother cells, shrinks in size, and clusters around the mother cells in a deeply staining irregular mass. Disintegration of the tapetum layer is increasingly rapid for 15 days, after which disintegration is almost complete and the fragments form an

incomplete layer around the mother cells. The layers of cells adjacent to the tapetum undergo similar but less rapid disintegration. These cells lose their contents and the walls collect around the periphery of the pollen sac. (Fig. 17.) The epidermis and the layer

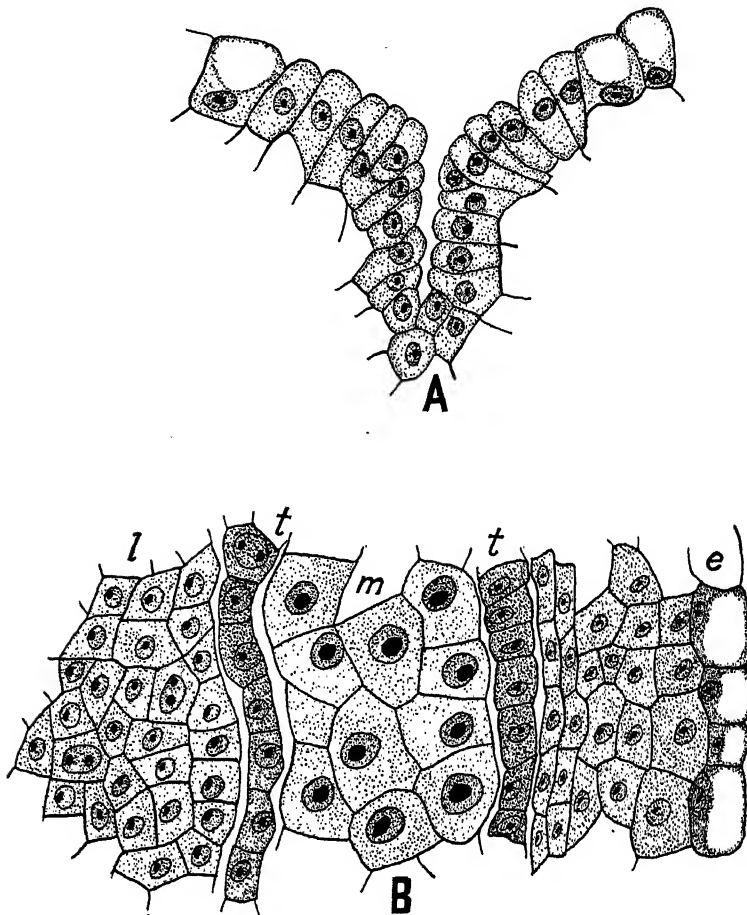


FIGURE 15.—A, Camera lucida drawing of cross section of a stomium of a mature anther; B, longitudinal section of a microsporangium showing the mature mother cells, *m*, just before rounding up; the tapetum cells, *t*, showing the first signs of disintegration; the cells between the loculi, *l*, and the epidermis, *e*.

of cells next to it do not disintegrate, but increase in radial diameter. The cells of the middle layers continue to disintegrate until the pollen is mature and the wasting away of the wall separating two loculi on either side of the anther allows them to fuse into one, and is a part of the process of dehiscence.

The mother cells do not increase in number after the tapetum cells reach full size (35 to 40 days before pollen shedding). As the tapetum cells disintegrate, the mother cells become separated and loosely fill

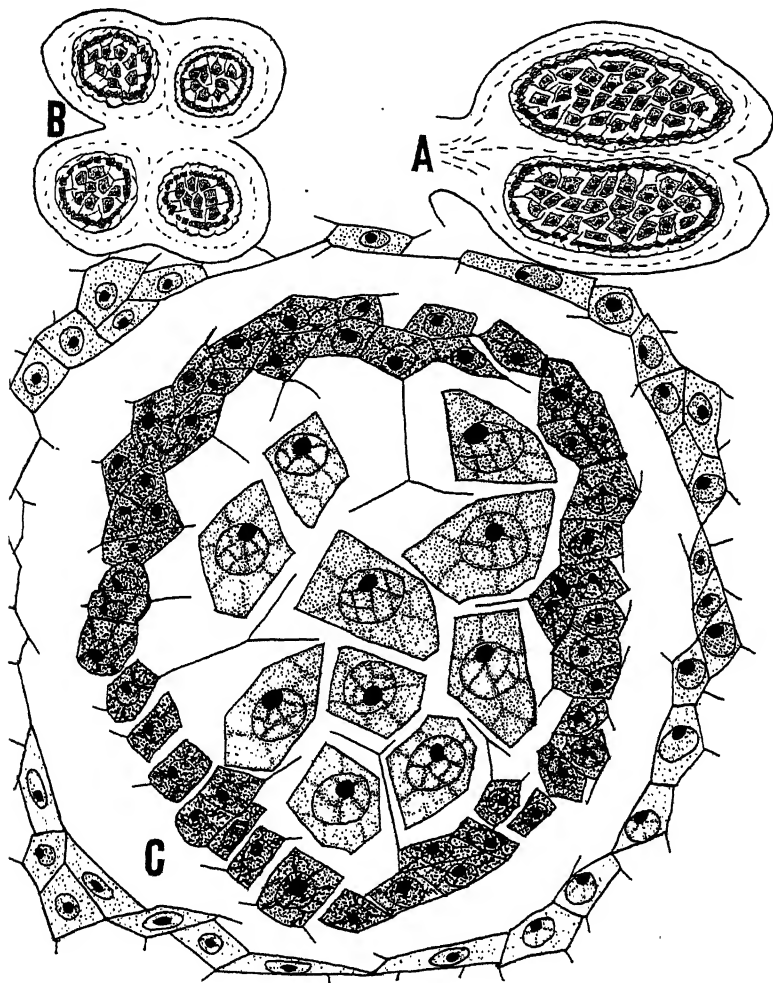


FIGURE 16.—A, Camera lucida drawing of longitudinal section of an anther of the Alley variety on April 1 showing the mother cells breaking apart and the tapetum cells rapidly disintegrating, $\times 110$; B, A shown in cross section; $\times 110$; C, enlargement of A; $\times 1003$. The Frottscher variety reached this same stage on April 15

the sporangium. Rounding up occurs about 21 days before pollen shedding and reduction division follows immediately.

Division of the mother cells and formation of tetrads apparently require only a few hours and the entire process seems to go to completion in an anther at a specific time on a single day. (Fig. 18.)

TETRAD

It is during the heterotypic and homotypic divisions that the first abnormal behavior has been observed. The appearance of all of the mother cells is normal, and they seem very uniform in cell contents, staining reaction, and size and shape. (Figs. 2, 14, and 19.) In the process of division various types of abnormalities occur. Dividing cells were found in the Frotscher variety April 26 at 9 a. m. Practically all steps in the process of heterotypic and homotypic divisions were present in a single anther. In the formation of new nuclei the organization of the chromatin results in nuclei of various sizes and

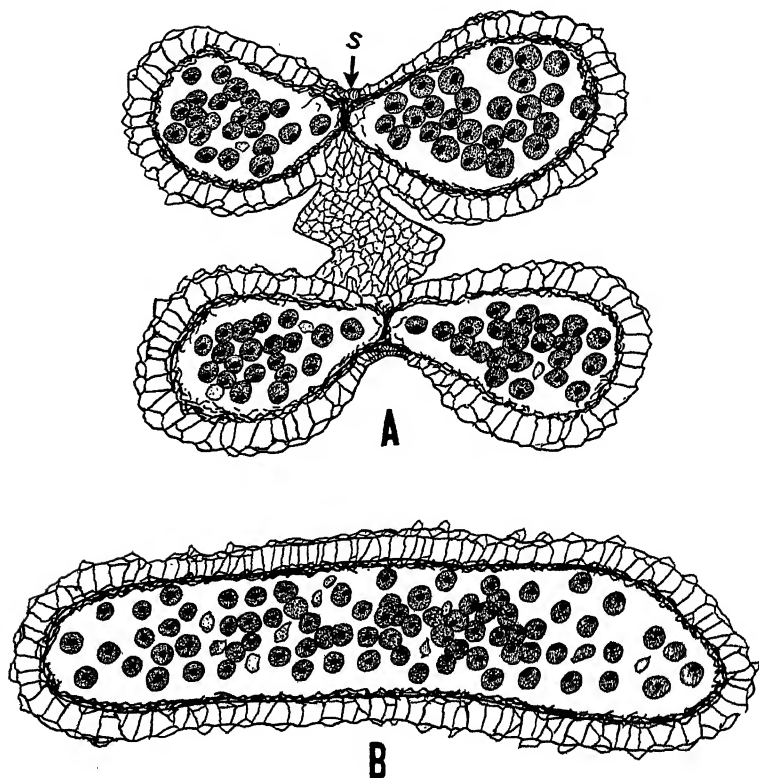


FIGURE 17.—Camera lucida drawing of cross section of anther just before dehiscence (A), and a tangential longitudinal section of the same (B); stomium at *s*. Stuart variety, May 13. $\times 110$

density as determined by the intensity of staining. Because of the minuteness of the chromosomes the number has not been definitely determined. In most cases the haploid number was found to be 12, in others only 10. In some cases where the nucleus was in the anaphase stage of the first division, one or two chromosomes were lagging slightly, but in some of the more advanced stages they had completely caught up with the other chromosomes.

Both the successive and simultaneous methods of division of the mother cells are common in pecans, producing bilateral and tetrahedral arrangements, respectively, of the microspores. (Figs. 20, and 21.) In the bilateral arrangement the spindles lie in the same or

in perpendicular planes, and no wall is formed between the successive divisions. The two arrangements are about equally common. No case has been observed where the four microspores were in a row within the tetrad wall, nor has a failure of four microspores to form been found. Small supernumerary microspores are often seen which, according to Shoemaker (19), may be the result of small nuclei formed from lagging chromosomes, or, according to Coulter and Chamberlain (?), from division of one or more members of the tetrad. Small microspores are especially numerous in lots of pollen containing a

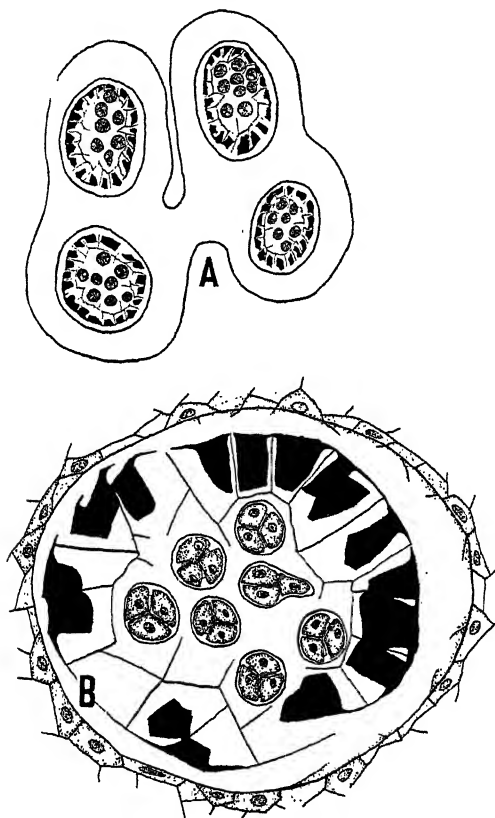


FIGURE 18.—A, Camera lucida drawing of cross section of an anther of the Frotcher variety on April 26 showing the tetrad stage with fragments of the tapetum cells; $\times 82$; B, enlargement of A; $\times 400$. Alley reached this stage on April 10

high percentage of defective grains and are almost always partially or totally void of cell contents. A few oversized grains were found, and the cell contents of most of them appeared normal. The normal number of pores is three, and variance is very rare with normal or undersized microspores; however, oversized microspores often have five or six pores.

At maturity some of the microspores are without nuclei and others without either nuclei or cytoplasm. The lack of protoplasmic contents is the basis of the lactic acid method of determining the percent-

age of defective pollen. By the use of this method defective grains were found in all lots examined, the number ranging from 0.3 to 81.7 per cent.

Flowers of the Beverage variety were found in the tetrad stage on April 24, and counts were made as follows: Four anthers taken from the base of a catkin contained 294, 373, 331, and 357 tetrads, respectively; and four anthers taken from the middle of the catkin contained 380, 380, 317, and 488 tetrads. Since each anther contains four pollen sacs, and each tetrad contains four microspores, the above figures also

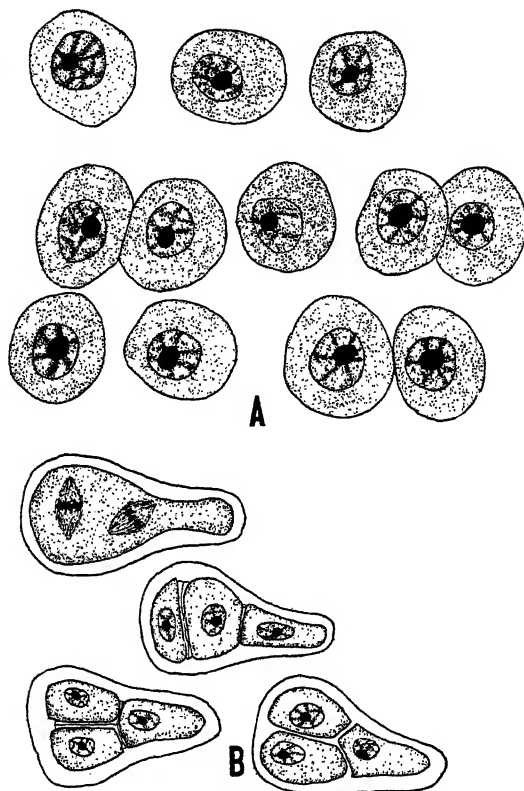


FIGURE 19.—A, Camera lucida drawing of mature mother cells that appear normal; B, mother cells with abnormal microspores. $\times 850$

represent the average number of pollen grains per pollen sac in the respective anthers, the general average being 365.

Liberation of the microspores (pollen grains) occurs from 15 to 20 days before the pollen is shed. Because of the disintegration of the tapetum and surrounding cells, the pollen sac is only partially filled with pollen. Upon liberation from the tetrad wall the grains wander independently within the pollen sac and rapidly enlarge in size, but as the cavity continues to enlarge, owing to the continued disintegration of the surrounding sterile cells, the pollen sac remains only partially filled.

DEHISCENCE OF ANTHERS

The fusion of the four pollen sacs into two loculi by the dissolution of the separating wall occurs only a few days before the opening of

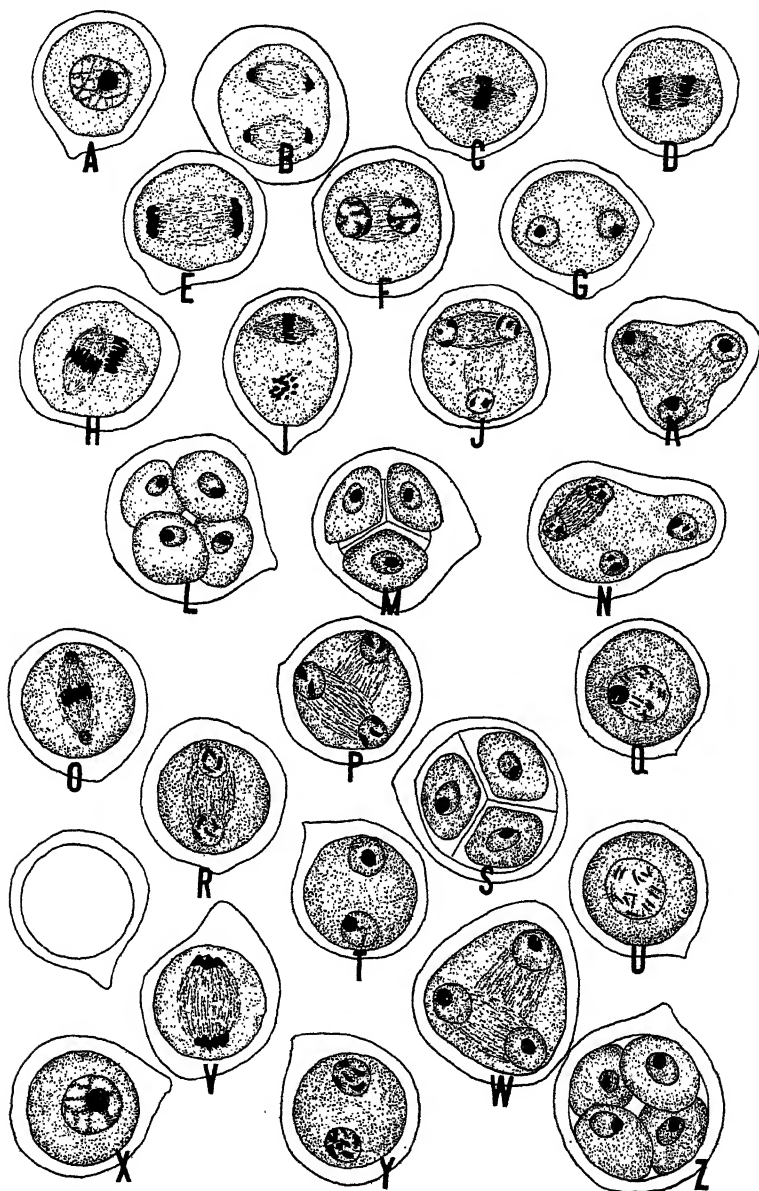


FIGURE 20.—Camera lucida drawings of stages of divisions of pollen mother cells at 9 a. m., April 26; Frottscher variety. Note formation of both bilateral and tetrahedral arrangements of microspores; all had reached the tetrad stage the following day. $\times 850$

the anther on either side by means of a stomium, with consequent liberation of pollen grains at maturity. (Figs. 15 and 17.) The anther wall is two cells thick, with fragments of one or more additional

layers. The opening of the anther is due to drying of the exterior or epidermal cells and consequent contraction of the outer in proportion to the inner surface. Reclosing of anthers occurs when moisture is

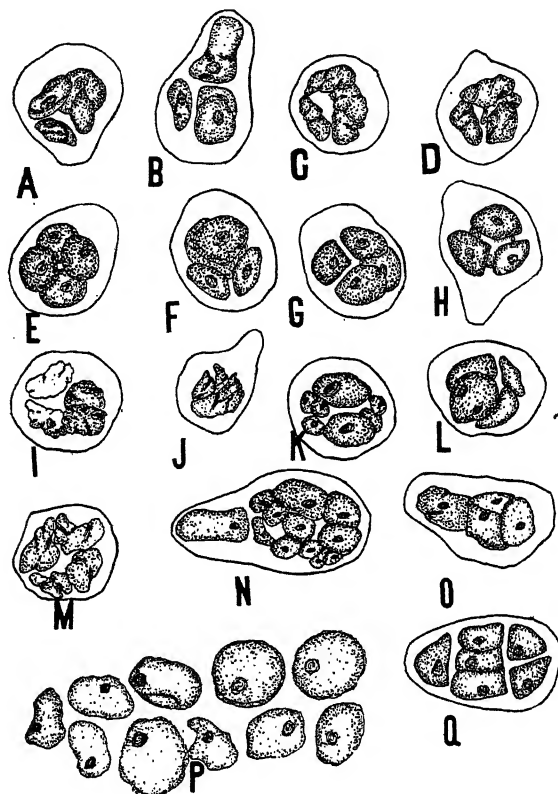


FIGURE 21.—Camera lucida drawings of tetrads and young pollen grains: E, F, G, and H appear normal; K, N, and Q contain more than four microspores; at P are shown pollen grains two days after liberation from the mother cell wall, some of the grains being defective. $\times 450$

restored. No opening takes place in an atmosphere more than about 85 per cent saturated. (Tables 2 and 3.)

TABLE 2.—Data on dehiscence of ripe anthers when placed in moist and dry air at different temperatures

Temperature	Humidity	Condition of anthers
3° C.....	Moist.....	Failed to dehisce.
	Dry.....	Dehisced in 5 days.
22° C.....	Moist.....	Failed to dehisce.
	Dry.....	Dehisced in 2 days.
23° C.....	Moist.....	Failed to dehisce.
	Dry.....	Dehisced in 18 hours.
32° C.....	Moist.....	Failed to dehisce.
	Dry.....	Dehisced in 6 hours.

TABLE 3.—Summary of data or pollen dissemination at various times of day in 26 varieties of pecans in the Georgia Experiment Station orchard

Date	6 p. m. to 6 a. m.				6 a. m. to 9 a. m.				9 a. m. to 12 m.				12 m. to 3 p. m.				3 p. m. to 6 p. m.			
	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter
1929	Per cent	° C.	Miles per hour	Grains	Per cent	° C.	Miles per hour	Grains	Per cent	° C.	Miles per hour	Grains	Per cent	° C.	Miles per hour	Grains	Per cent	° C.	Miles per hour	Grains
Apr. 18.---	68	66	1	11	75	67	12	24	65	72	8	17	57	77	4	18	80	66	3	39
Apr. 19.---	60	86	3	32	65	74	6	24	61	77	11	54	61	77	7	71	80	60	3	10
Apr. 20.---	70	51	10	7	87	69	15	6	71	77	15	61	69	72	2	60	70	10	2	17
Apr. 21.---	75	67	2	7	87	69	15	31	65	77	15	84	68	82	3	50	67	3	10	7
Apr. 22.---	65	78	3	65	67	64	12	27	60	67	6	61	58	72	11	69	73	3	3	74
Apr. 23.---	64	72	4	4	67	64	4	27	60	72	7	128	60	72	5	38	64	4	4	37
Apr. 24.---	64	72	4	4	67	64	4	27	60	72	6	128	60	72	5	38	64	4	4	37
Apr. 25.---	77	63	8	26	81	64	9	18	64	73	11	10	60	80	14	67	73	77	8	129
Apr. 26.---	77	63	8	5	81	64	9	18	64	73	11	10	60	80	14	67	73	77	8	129
Apr. 27.---	59	83	4	10	83	72	12	63	78	64	4	2	75	63	8	0	80	59	4	6
Apr. 28.---	62	68	3	13	83	72	12	63	78	64	4	2	75	68	5	17	80	62	4	6
Apr. 29.---	73	50	3	10	74	63	7	16	70	75	4	37	76	72	8	13	98	61	4	0
Apr. 30.---	64	90	4	0	78	74	6	0	81	77	3	24	83	77	6	8	90	78	3	4
May 1.---	70	81	3	0	78	74	6	0	81	77	2	24	83	80	4	10	90	64	4	1
May 2.---	49	90	10	0	72	65	11	4	69	67	9	9	77	58	12	16	81	70	3	0
May 3.---	58	75	2	0	72	62	5	0	69	62	12	9	77	58	12	16	81	70	3	0
May 4.---	58	75	2	0	72	62	5	0	69	62	12	9	77	58	12	16	81	70	3	0
May 5.---	64	65	4	0	60	77	5	1	86	62	7	2	68	62	10	1	90	49	2	0
May 6.---	72	78	3	0	85	73	8	1	75	73	9	1	86	62	10	2	65	64	4	0
May 7.---	79	69	3	0	90	72	9	0	82	76	9	1	71	80	4	1	75	72	3	0
May 8.---	69	86	3	2	90	68	10	17	87	75	10	2	84	73	11	4	69	87	3	0
May 9.---	64	93	2	0	95	62	8	0	90	66	6	0	93	64	6	0	93	64	2	0
Average..	66.6	76.2	3.9	11.9	79.8	67.3	8.4	12.4	75.5	71.4	7.9	21.8	72.8	72.3	7.2	32.0	76.7	66.6	4.2	20.2
1928																				
May 2.---	58	77	1	7	36	70	1	0	33	76	5	34	45	80	3	41	55	66	2	43
May 3.---	42	77	4	52	42	82	5	40	41	85	5	9	85	85	4	9	50	74	2	4
May 4.---	59	76	10	196	60	82	6	22	35	80	2	35	41	80	2	35	77	72	2	13
May 5.---	59	76	10	196	60	82	6	22	35	80	2	35	41	80	2	35	77	72	2	13
May 6.---	59	76	10	196	60	82	6	22	35	80	2	35	41	80	2	35	77	72	2	13
May 7.---	37	86	3	136	70	62	12	16	69	82	6	50	45	81	2	81	59	76	4	125
May 8.---	49	86			85	52			98	49										
May 9.---	44	93	6	2	72	59	8	1	38	72	4	50	40	57	9	3	87	52	4	4
May 10.---	53	75	3	6	52	71	3	9	38	81	6	16	26	75	6	88	44	66	2	21
May 11.---	60	68	2	10	39	78	6	7	32	84	2	4	30	88	7	28	59	69	0	17

TABLE 3.—Summary of data on pollen dissemination at various times of day in 26 varieties of pecans in the Georgia Experiment Station orchard—Continued

Date	6 p. m. to 6 a. m.				6 a. m. to 9 a. m.				9 a. m. to 12 m.				12 m. to 3 p. m.				3 p. m. to 6 p. m.			
	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter	Relative humidity	Temperature	Wind velocity	Pollen per square centimeter
1928	Per cent	° C.	Miles per hour	Grains	Per cent.	° C.	Miles per hour	Grains	Per cent	° C.	Miles per hour	Grains	Per cent	° C.	Miles per hour	Grains	Per cent	° C.	Miles per hour	Grains
May 12--	60	78	8	10	71	67	1	8	62	67	12	10	55	69	7	19	48	70	4	38
May 13--	58	81	7	14	74	62	10	10	60	71	11	8	53	72	8	4	64	62	5	2
May 14--	58	83	6	2	43	67	8	4	42	73	10	5	48	77	8	6	59	68	6	2
May 15--	60	81	4	6	65	66	5	1	41	76	7	7	47	77	7	10	56	68	4	9
May 16--	60	81	1	0	72	70	3	20	65	73	8	14	69	72	7	151	72	69	2	48
May 17--	64	80	4	1	81	72	2	90	67	78	1	122	91	74	2	3	51	69	1	6
May 18--	68	90	2	1	74	79	0	32	64	80	0	486	81	86	7	381	59	77	2	57
May 19--	69	90	1	4	78	73	0	1	73	81	7	121	98	78	3	90	78	73	4	8
May 20--	67	90	4	1	82	73	7	7	71	78	8	4	67	78	6	16	70	75	3	10
May 21--	65	95	4	0	83	69	6	0	67	77	6	1	68	76	6	5	70	75	1	1
May 22--	65	95	2	0	85	66	1	0	77	71	4	0	---	---	---	---	---	---	---	---
Average--	59.7	82.1	3.6	12.8	65.8	69.2	5.6	24.5	56.3	75.5	6.3	55.7	58.2	75.6	5.5	51.4	59.2	69.9	2.5	28.0
1927																				
Apr. 22--				1				6				7				3				0
Apr. 23--				0				0				1				2				1
Apr. 24--				1				0				3				2				5
Apr. 25--				0				0				0				0				0
Apr. 26--				2				1				0				1				4
Apr. 27--				6				3				2				5				13
Apr. 28--				6				3				2				18				55
Apr. 29--				51				51				82				62				16
May 4--				17				17				15				10				10
May 5--				3				1				15				26				14
May 6--				3				1				1				14				14
May 7--				19				12				1				14.3				12.7
Average--				4				8.5				12.4				14.3				12.7

An idea of the maturity of pollen can be had from the stiffness of the catkin. A catkin that would not shed pollen within 48 hours is relatively stiff; one that would normally shed within 12 hours in warm, sunny weather is limber. Within two days after pollen is shed the catkin becomes dry, very stiff, and falls to the ground.

The stage of maturity of pollen can best be determined by the color of the anthers. From the time of emerging from the bud scales until 48 hours before pollen would normally shed under suitable conditions, the anthers are the same color as the bracts and leaves. After this time, however, the green color gradually disappears and the anthers take on more and more of the orange-yellow color of the pollen. When fully ripe the anther is pale greenish yellow. If conditions are suitable, dehiscence will occur immediately, but if the temperature is too low, or the humidity above about 85 per cent, the catkin may remain for five days or more without falling to the ground.

The rapidity of opening of ripe anthers depends somewhat on temperature but largely on the relative humidity of the air. On a warm, sunny day a single anther will completely shed its contents in an hour after opening begins. Due to variations in catkins, twigs, trees, and varieties under the same conditions, a single catkin will shed pollen for 2 days, a single tree will shed pollen for 5 or 6 days, a single variety will furnish pollen for 10 or 12 days, and a collection of three or more varieties of each group will furnish pollen for about 3 weeks.

If wilting was produced very rapidly by the high temperatures immediately after the catkins were removed from the tree, opening did not occur. Likewise, if the relative humidity was above 82 per cent the anthers remained closed. Partly or completely open anthers closed in a few minutes when subjected to a relative humidity of more than 90 per cent. Catkins that had fallen from the tree and become dry reabsorbed moisture and the anthers closed in the presence of high humidity.

Records taken in the orchard at 3-hour intervals showed that the time of most rapid shedding of pollen varied from day to day, depending on the temperature and the relative humidity of the air. No shedding occurred when the relative humidity was above 85 per cent. On some days the peak was reached before 9 a. m. On other days it was delayed until after 3 p. m., but it never occurred between 6 p. m. and 6 a. m. The temperature, relative humidity, and wind-velocity data in Table 3 were taken at the end of the time of exposure of the slides, and in some cases do not accurately indicate the conditions that prevailed during the period of exposure. The apparatus for catching pollen (fig. 1, A) remained in the same place throughout the pollen-shedding season for three years.

In 1928, in the midst of the pollen-shedding season, a period of 64 hours elapsed with no shedding because of rain. Three 24-hour periods occurred during the same season when no pollen was shed. As a result of low temperatures and high humidity in 1929, not more than 5 days out of 21 covered in the pollen-shedding season were really favorable for pollen dissemination. The relative humidity and temperature reached during clear nights with a gentle breeze were not sufficient to cause anthers already open to close, but they did prevent the further opening of anthers. A dew or rain which caused

the relative humidity to rise above 90 per cent caused all anthers to close and delayed shedding until several hours after sunrise on the following morning.

Since very high or very low temperatures do not occur at the time that pecans shed their pollen, the influence of temperature and wind velocity is chiefly indirect in regulating the relative humidity of the air. Though there is no opening of anthers during damp, rainy weather, immature anthers continue to reach the stage of maturity, therefore a period of high relative humidity followed by a prolonged period of low relative humidity is accompanied by the shedding of a very large quantity of pollen for several hours.

Close observation and careful records kept for a number of years (30) show that the length of the receptive period of the pistillate flowers is as responsive to conditions of temperature and humidity as that of staminate flowers. The same conditions which delay the shedding of pollen also prolong the receptive period of the stigmas. Trees on which the stigmas became receptive at a time unfavorable for pollen shedding and favorable for pollen germination did not have a heavy May "drop."

The effect of high humidity is mainly that of retardation. Instances have been observed in which stigmatic surfaces dried as much in 24 hours under dry, windy conditions as they did in two weeks of damp, cloudy weather. During a prolonged drought the susceptibility of the stigmatic surfaces to rapid drying increases as the receptive stage is approached, and on the second day after becoming receptive the roughened surface darkens, shrinks, and dies.

In rainy weather the receptive stage is reached very gradually, and after about 10 days the stigmatic surfaces show symptoms of drying. If cloudy weather continues the stigmas do not become completely dry for about 15 days. The normal period of receptivity of a single flower is about five days.

Observations of about 25 varieties for seven years show that during rainy seasons there are usually enough sunny days to effect pollination; and that dry weather, though optimum for pollen dissemination often precedes a heavy May drop. Data in this paper indicate that the degree of humidity optimum for pollen shedding is not sufficient to cause germination of pollen; also a low relative humidity markedly reduces the viability of the pollen.

Table 4 shows the amount of pollen caught on greased slides at various distances from pollen-shedding trees. The apparatus was always kept on the leeward side of the trees. The difference between the amount of pollen caught at a height of 10, 20, or 30 feet is insignificant. The amount of pollen that will be blown by the wind depends on the humidity, temperature, and velocity of the circulating air. The path of the pollen from a small group of trees is narrow at a given time, but the horizontal direction of air currents is likely to vary greatly during the 10 or 12 days of pollen shedding of a single variety. Throughout the southeastern part of the United States winds from the east and south are somewhat moist and are not as conducive to pollen shedding as winds from the west and northwest.

TABLE 4.—Amount of pollen blown by a 2 to 5 mile-per-hour wind from a 20-year-old pecan orchard of Mobile, Stuart, and Teche varieties, 1927 and 1928

Distance from orchard	Height from ground	Year and time of day	Pollen per square centimeter	Day after pollen shedding began
	<i>Feet</i>	1927	<i>Grains</i>	
500 feet.....	10	2 p. m. to 5 p. m.....	70.7	First.
Do.....	20	do.....	80.7	Do.
Do.....	30	do.....	92.6	Do.
Do.....	10	5 p. m. to 7 a. m.....	0.8	Do.
Do.....	20	do.....	3.7	Do.
Do.....	30	do.....	4.5	Do.
800 feet.....	10	8 a. m. to 11 a. m.....	137.8	Second.
Do.....	20	do.....	162.5	Do.
Do.....	30	do.....	156.5	Do.
Do.....	10	do.....	3.8	Fourth.
Do.....	20	do.....	5.2	Do.
Do.....	30	do.....	5.6	Do.
1,000 feet.....	10	11 a. m. to 2 p. m.....	19.3	Do.
Do.....	20	do.....	28.0	Do.
Do.....	30	do.....	28.3	Do.
Do.....	10	2 p. m. to 5 p. m.....	8.3	Do.
Do.....	20	do.....	16.6	Do.
Do.....	30	do.....	15.5	Do.
Do.....	10	5 p. m. to 7 a. m.....	13.1	Do.
Do.....	20	do.....	21.4	Do.
Do.....	30	do.....	19.1	Do.
Do.....	10	7 a. m. to 11 a. m.....	11.1	Fifth.
Do.....	20	do.....	13.2	Do.
Do.....	30	do.....	13.5	Do.
Do.....	10	11 a. m. to 2 p. m.....	64.9	Do.
Do.....	20	do.....	107.1	Do.
Do.....	30	do.....	134.7	Do.
Do.....	10	2 p. m. to 5 p. m.....	28.6	Do.
Do.....	20	do.....	44.8	Do.
Do.....	30	do.....	39.7	Do.
		1928		
3,000 feet.....	20	2 p. m. to 2 p. m. ^a	10.6	First.
Do.....	30	do.....	8.4	Second.
Do.....	10	do.....	10.6	Third.
Do.....	20	do.....	13.3	Fourth.
Do.....	30	do.....	13.6	Fifth.

^a 24 hours.

TABLE 5.—Dates on which pollen was shed and on which pistillate flowers were receptive of commercial varieties of pecans in leading pecan-growing centers of southeastern United States

1926

Location and variety of pecan	Pollen shedding		Pistils receptive		Group	Self-pollinated
	Began	Ended	Began	Ended		
Tifton, Ga.:						
Alley.....	Apr. 27	-----	Apr. 30	-----	1	Yes.
Big Z.....	Apr. 29	-----	Apr. 28	-----	2	Yes.
Bradley.....	do.....	-----	Apr. 26	-----	2	Yes.
Curtis.....	Apr. 28	-----	Apr. 28	-----	2	Yes.
Delmas.....	May 1	-----	Apr. 27	-----	2	Yes.
Frotscher.....	May 4	-----	Apr. 29	-----	2	Yes.
Mobile.....	Apr. 26	-----	Apr. 28	-----	1	Yes.
Moneymaker.....	May 1	-----	Apr. 27	-----	2	Yes.
Moore.....	Apr. 26	-----	Apr. 26	-----	1	Yes.
Nelson.....	do.....	-----	Apr. 27	-----	1	Yes.
Pabst.....	do.....	-----	Apr. 28	-----	1	Yes.
President.....	Apr. 23	-----	Apr. 30	-----	2	Yes.
Schley.....	Apr. 29	-----	Apr. 28	-----	2	Yes.
Stuart.....	Apr. 30	-----	Apr. 29	-----	2	Yes.
Summers.....	Apr. 26	-----	Apr. 28	-----	1	Yes.
Teche.....	Apr. 30	-----	do.....	-----	2	Yes.
Van Deman.....	Apr. 28	-----	Apr. 25	-----	2	Yes.

TABLE 5.—*Dates on which pollen was shed and on which pistillate flowers were receptive of commercial varieties of pecans in leading pecan-growing centers of southeastern United States—Continued*

1927

Location and variety of pecan	Pollen shedding		Pistils receptive		Group	Self-pollinated
	Began	Ended	Began	Ended		
Tifton, Ga.:						
Alley.....	Apr. 21	-----	Apr. 20	-----	1	No.
Big Z.....	Apr. 29	-----	May 7	-----	2	No.
Bradley.....	Apr. 21	-----	Apr. 28	-----	2	No.
Curtis.....	Apr. 22	-----	May 3	-----	2	No.
Delmas.....	Apr. 26	-----	Apr. 29	-----	2	Yes.
Frotscher.....	Apr. 12	-----	Apr. 18	-----	2	Yes.
Mobile.....	Apr. 29	-----	May 6	-----	1	No.
Moneymaker.....	Apr. 18	-----	Apr. 21	-----	2	Yes.
Moore.....	Apr. 21	-----	Apr. 26	-----	1	Yes.
Nelson.....	Apr. 9	-----	Apr. 22	-----	1	No.
Pabst.....	Apr. 18	-----	Apr. 29	-----	1	No.
President.....	Apr. 21	-----	Apr. 26	-----	2	Yes.
Schley.....	do	-----	Apr. 29	-----	2	No.
Success.....	do	-----	do	-----	1	No.
Teche.....	do	-----	do	-----	2	No.
Van Deman.....	Apr. 26	-----	do	-----	2	Yes.

1928

Tifton, Ga.:						
Alley.....	Apr. 30	-----	May 5	-----	1	Yes.
Big Z.....	Apr. 28	-----	May 7	-----	2	No.
Bradley.....	do	-----	May 5	-----	2	No.
Mobile.....	Apr. 30	-----	May 3	-----	1	Yes.
Moneymaker.....	do	-----	May 5	-----	2	Yes.
Moore.....	Apr. 28	-----	May 2	-----	1	Yes.
Nelson.....	Apr. 21	-----	Apr. 23	-----	1	No.
Pabst.....	Apr. 30	-----	May 2	-----	1	Yes.
President.....	do	-----	May 5	-----	2	Yes.
Schley.....	Apr. 28	-----	May 2	-----	2	Yes.
Success.....	do	-----	May 5	-----	1	No.
Teche.....	Apr. 30	-----	do	-----	2	Yes.

1929

Tifton, Ga.:						
Alley.....	-----	Apr. 20	Apr. 22	-----	1	No.
Big Z.....	Apr. 22	-----	-----	Apr. 20	2	No.
Bradley.....	do	-----	-----	-----	2	No.
Curtis.....	Apr. 24	-----	-----	Apr. 21	2	No.
Delmas.....	-----	Apr. 18	-----	Apr. 20	2	Yes.
Frotscher.....	Apr. 23	-----	-----	Apr. 21	2	No.
Mobile.....	-----	Apr. 19	Apr. 22	-----	1	No.
Moneymaker.....	Apr. 21	-----	Apr. 21	-----	2	Yes.
Moore.....	-----	Apr. 18	Apr. 20	-----	1	No.
Nelson.....	-----	Apr. 22	Apr. 25	-----	1	No.
Pabst.....	-----	Apr. 21	Apr. 24	-----	1	No.
President.....	Apr. 20	-----	-----	Apr. 20	2	Yes.
Rome.....	-----	Apr. 18	Apr. 22	-----	1	No.
Schley.....	Apr. 21	-----	Apr. 21	-----	2	Yes.
Stuart.....	Apr. 24	-----	-----	Apr. 21	2	No.
Success.....	-----	Apr. 18	Apr. 22	-----	1	No.
Summers.....	-----	Apr. 19	Apr. 21	-----	1	No.
Teche.....	Apr. 23	-----	-----	Apr. 20	2	No.
Van Deman.....	do	-----	Apr. 22	-----	2	Yes.
Williams.....	do	-----	Apr. 23	-----	2	Yes.
Cordele, Ga.:						
Frotscher.....	Apr. 20	-----	Apr. 20	-----	2	Yes.
Mobile.....	Apr. 20	-----	-----	Apr. 29	1	No.
Moore.....	Apr. 18	-----	Apr. 20	-----	1	Yes.
Nelson.....	-----	Apr. 18	Apr. 21	-----	1	No.
Schley.....	Apr. 20	-----	Apr. 20	-----	2	Yes.
Stuart.....	do	-----	do	-----	2	Yes.
Van Deman.....	Apr. 23	-----	-----	Apr. 19	2	No.
Altany, Ga.:						
Schley.....	Apr. 22	-----	-----	Apr. 25	2	Yes.
Stuart.....	do	-----	-----	Apr. 26	2	Yes.
Van Deman.....	do	-----	-----	Apr. 25	2	Yes.

TABLE 5.—*Dates on which pollen was shed and on which pistillate flowers were receptive of commercial varieties of pecans in leading pecan-growing centers of southeastern United States—Continued*

Location and variety of pecan	Pollen shedding		Pistils receptive		Group	Self-pollinated
	Began	Ended	Began	Ended		
Monticello, Fla.:						
Curtis.....	Apr. 21	-----	Apr. 19	Apr. 25	2	Yes.
Frotscher.....	Apr. 24	-----	-----	Apr. 22	2	No.
Mahan.....	Apr. 21	-----	-----	Apr. 23	2	Yes.
Moore.....	-----	Apr. 18	Apr. 21	-----	1	No.
Russell.....	Apr. 20	-----	-----	Apr. 22	2	Yes.
Schley.....	Apr. 22	-----	Apr. 22	-----	2	Yes.
Stuart.....	Apr. 24	-----	Apr. 23	-----	2	Yes.
Teche.....	Apr. 22	-----	Apr. 21	-----	2	Yes.
Experiment, Ga.:						
Alley.....	Apr. 18	Apr. 24	Apr. 28	May 8	1	No.
Appomattox.....	Apr. 28	May 3	Apr. 22	May 1	2	Yes.
Beverage.....	Apr. 18	Apr. 24	do	May 6	1	Yes.
Bradley.....	Apr. 29	May 7	do	Apr. 26	2	No.
Centennial.....	Apr. 18	Apr. 25	Apr. 25	May 3	1	No.
Curtis.....	Apr. 30	May 12	Apr. 23	May 2	2	Yes.
Delmas.....	do	May 7	Apr. 21	do	2	Yes.
Indiana.....	May 6	May 12	Apr. 30	May 8	2	Yes.
Jerome.....	Apr. 21	Apr. 30	Apr. 24	May 9	1	Yes.
Mantura.....	Apr. 18	Apr. 23	Apr. 22	May 1	1	Yes.
Mobile.....	do	Apr. 24	-----	-----	1	No.
Moore.....	do	Apr. 23	Apr. 22	Apr. 30	1	Yes.
Nelson.....	do	Apr. 24	do	do	1	Yes.
Pabst.....	Apr. 20	do	-----	-----	1	No.
Randal.....	Apr. 18	Apr. 27	-----	-----	1	No.
Robson.....	do	Apr. 24	Apr. 23	May 3	1	Yes.
Rome.....	Apr. 20	Apr. 30	Apr. 24	May 9	1	Yes.
San Saba.....	Apr. 21	Apr. 26	do	May 8	1	Yes.
Schley.....	Apr. 29	May 7	Apr. 22	May 3	2	Yes.
Stuart.....	do	May 8	do	May 7	2	Yes.
Success.....	Apr. 22	Apr. 26	Apr. 25	May 9	1	Yes.
Teche.....	Apr. 29	May 7	Apr. 20	Apr. 24	2	No.
Van Deman.....	May 1	May 8	Apr. 21	Apr. 28	2	No.
Thomasville, Ga.:						
Frotscher.....	Apr. 22	-----	-----	Apr. 21	2	No.
Mobile.....	-----	Apr. 18	Apr. 22	-----	2	No.
Stuart.....	Apr. 22	-----	-----	Apr. 24	2	Yes.
Schley.....	do	-----	-----	do	2	Yes.
Athens, Ga.:						
Alley.....	-----	May 3	May 6	May 13	1	No.
Delmas.....	May 6	May 15	May 3	May 11	2	Yes.
Frotscher.....	Apr. 31	May 6	May 1	May 9	2	Yes.
Jerome.....	-----	do	May 5	May 13	1	Yes.
Mobile.....	-----	do	-----	-----	1	No.
Nelson.....	-----	May 3	-----	-----	1	No.
Pabst.....	May 1	May 9	May 6	May 13	1	Yes.
Success.....	May 7	May 13	-----	-----	1	Yes.
Schley.....	May 6	do	May 2	May 10	2	Yes.
Stuart.....	do	May 14	May 3	May 12	2	Yes.
Teche.....	May 7	do	May 2	May 11	2	Yes.
Van Deman.....	May 6	May 15	Apr. 30	May 9	2	Yes.
Raleigh, N. C.:						
Appomattox.....	May 3	do	Apr. 29	May 10	2	No.
Bradley.....	May 6	do	-----	May 1	2	Yes.
Curtis.....	May 10	May 23	May 1	May 19	2	Yes.
Delmas.....	May 9	May 19	May 3	May 11	2	Yes.
Frotscher.....	May 7	May 21	-----	May 3	2	No.
Georgia.....	-----	May 6	May 5	May 23	1	Yes.
Krakezy.....	-----	May 1	do	May 21	1	No.
Louisiana.....	May 6	May 15	-----	May 8	2	Yes.
Manture.....	-----	May 5	May 5	May 18	1	No.
Mobile.....	-----	May 1	May 1	May 23	1	No.
Moneymaker.....	May 5	May 16	-----	May 3	2	No.
Pabst.....	May 1	May 15	May 3	May 23	1	Yes.
President.....	May 10	May 23	May 1	May 11	2	Yes.
Russel.....	May 9	May 19	May 3	May 10	2	Yes.
Schley.....	May 10	May 23	May 1	May 17	2	Yes.
Sovereign.....	-----	May 3	May 3	May 24	1	No.
Stuart.....	May 5	May 21	May 1	May 13	2	Yes.
Success.....	do	May 18	May 3	May 24	1	Yes.
Teche.....	May 7	May 22	May 1	May 15	2	Yes.
Van Deman.....	May 10	May 21	do	May 10	2	No.

Table 5 contains incomplete data on dates of beginning and ending of pollen shedding and beginning and ending of receptivity of stigmas of 142 varieties from eight pecan-producing sections in these States.

In varieties of Group 1 there were 31 instances of homogamy and 25 instances of complete dichogamy, all of the latter being protandrous. In varieties of Group 2 there were 57 instances of homogamy and 29 instances of dichogamy—11 instances of protandry and 18 instances of protogyny.

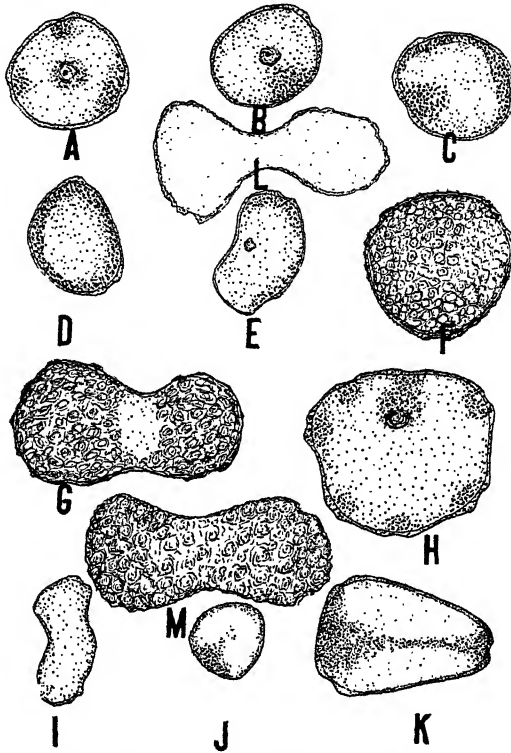


FIGURE 22.—Camera lucida drawings of pollen grains in lactic acid mounts: A, B, C, D, E, H, I, J, and K contain a small amount of finely granular cytoplasm; G and M are elongated grains; H, a grain with seven pores; F, a normal grain. $\times 850$

EXAMINATION OF POLLEN

The writer has confirmed Stuckey's (23) observation that "in size, shape and general character, the pollen of the two groups of varieties of pecans differ almost none." However, the grains are not "rather flattened," as he described them.

Dry pollen grains from a single variety vary slightly in size and shape, are sculptured, and uniformly pale yellow. They are spherical but become shrunken immediately after shedding, and when exposed to very dry air the shrinking increases. Fresh pollen taken from an atmosphere 80 per cent saturated at 21°C . and placed in an oven at 32° lost 5.5 per cent moisture; when placed in an oven at 60° it lost 10 per cent. Dry grains containing no protoplasm can not be distinguished from those with protoplasm. Though the increased amount of shrinkage which accompanies the absence of protoplasm

may serve as a means of identifying certain defective grains, many abnormal grains appear normal when dry.

Normal pollen grains mounted in distilled water swell immediately and turn dark. Grains without protoplasm fail to swell, and grains containing protoplasm in less than the normal amount swell so that they are indistinguishable from normal grains.

When mounted in lactic acid, normal grains become light cream in color and spherical in outline, and assume in one minute an appearance distinct from defective grains. The latter either fail to swell and remain opaque, or swell and become almost transparent. The

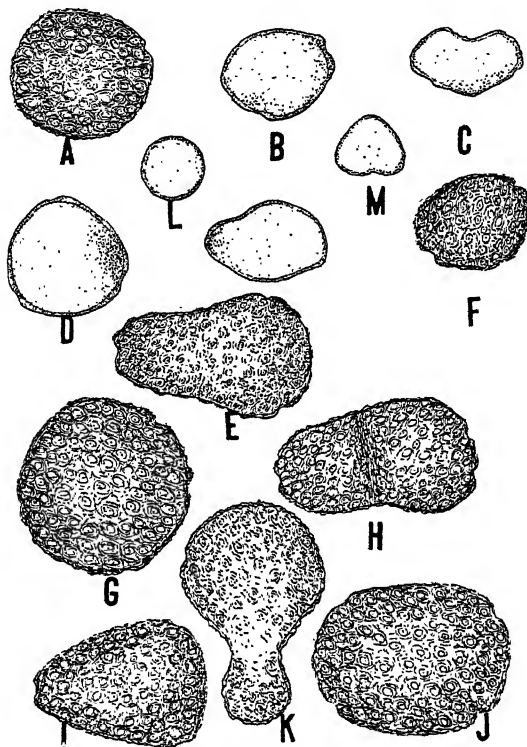


FIGURE 23.—Camera lucida drawings of pollen grains in lactic acid mounts: A, Normal grain with coarsely granular cytoplasm (Jerome); B, C, D, L, and M contain no cytoplasm (Jerome); F, a grain of less than normal size (Jerome); E, G, H (Jerome); I, K (Beverage), grains enlarged, abnormally shaped. $\times 800$

lack of normal protoplasmic content in certain grains is clearly evident. Some grains are without either protoplasm or pores, indicating that abnormality began early in the development; others contain a small amount of protoplasm and one or more pores; still others have the normal number of pores and swell normally, but the protoplasm granules are very fine and sufficiently different from normal grains to be termed defective.

A single pollen grain observed in lactic acid amount is spherical with a roughened surface. When the percentage of normal grains is high they are extremely uniform in size, but if the percentage of

defective grains is high there are numerous large, small, and odd-shaped grains. (Figs. 22 and 23.) A normal grain is about 50 microns in diameter (23) with a wall about 2 microns in thickness. There is no appreciable difference in the size of pollen of any of the varieties studied except variations due to a large amount of defective pollen.

PERCENTAGE OF DEFECTIVE POLLEN

Table 6 contains data on the amount of defective pollen found when 169 lots were examined by the lactic acid method. The total number of grains examined was 169,000.

TABLE 6.—Percentage of defective pollen as found by counting 1,000 grains in lactic acid mounts, 1928 and 1929 *

1928

Pecan variety and vigor	Average defective grains	Percentage of defective grains found at position indicated			Pecan variety and vigor	Average defective grains	Percentage of defective grains found at position indicated		
		Apex	Middle	Base			Apex	Middle	Base
Alley.....	19.5	-----	-----	-----	Pabst:	-----	-----	-----	-----
Atlanta.....	3.0	3.7	2.5	2.9	Vigorous.....	4.5	6.7	2.9	4.0
Beverage.....	6.7	-----	-----	-----	Nonvigorous.....	4.4	-----	-----	-----
Bradley.....	8.3	10.2	8.9	6.3	Randal.....	5.9	-----	-----	-----
Centennial.....	35.1	35.5	34.5	37.1	Robson.....	7.1	-----	-----	-----
Curtis.....	6.3	8.6	6.7	4.1	Rome.....	29.6	-----	-----	-----
Delmas.....	5.4	4.4	7.6	4.1	San Saba:	-----	-----	-----	-----
Frotscher.....	7.9	9.2	3.1	6.3	Vigorous.....	10.3	14.7	5.4	11.3
Indiana.....	6.4	6.0	7.3	6.0	Nonvigorous.....	13.4	-----	-----	-----
Jerome:	-----	-----	-----	-----	Schley.....	14.3	12.8	16.0	14.0
Vigorous.....	27.2	32.4	37.1	42.2	Stuart.....	46.6	81.7	27.6	20.6
Nonvigorous.....	35.2	31.6	33.5	40.5	Teche.....	7.5	8.0	5.1	9.4
Mantura.....	71.2	-----	-----	-----	Unknown.....	13.1	12.2	9.4	17.6
Moneymaker.....	23.8	10.5	44.8	16.3	Van Deman.....	29.2	35.6	44.5	37.5
Moore.....	20.2	-----	-----	-----	Wauchenah.....	5.8	5.7	6.3	5.3
Nelson.....	20.2	-----	-----	-----					

1929

Pecan variety	Location	Vigor	Average defective grains	Percentage of defective grains found at position indicated		
				Apex	Middle	Base
Alley.....	Experiment, Ga.....	Medium.....	10.2	-----	-----	-----
Appomattox.....	do.....	do.....	3	-----	-----	-----
Beverage.....	do.....	do.....	9	1.4	1.4	0
Bradley.....	do.....	do.....	6.9	7.7	4.4	8.5
Centennial.....	do.....	do.....	31.7	-----	-----	-----
Curtis.....	do.....	do.....	4.6	-----	-----	-----
Delmas.....	do.....	Vigorous.....	7.0	-----	-----	-----
Ga. Giant.....	Tifton, Ga.....	do.....	10.4	-----	-----	-----
Indiana.....	Experiment, Ga.....	Medium.....	3.3	4.3	2.3	4.4
Jerome.....	do.....	do.....	3.3	-----	-----	-----
Mahan.....	Monticello, Fla.....	Vigorous.....	12.0	-----	-----	-----
Mantura.....	Experiment, Ga.....	Medium.....	20.0	-----	-----	-----
McAlister.....	do.....	do.....	8.2	-----	-----	-----
Mobile.....	do.....	do.....	1.1	8	-----	1.4
Do.....	Cordele, Ga.....	do.....	8.4	-----	-----	-----
Do.....	Miner, Ga.....	Vigorous.....	1.7	-----	-----	-----
Do.....	Barnsville, Ga.....	do.....	3.7	-----	-----	-----
Do.....	do.....	do.....	2.6	-----	-----	-----
Do.....	do.....	do.....	8.0	-----	-----	-----
Do.....	do.....	Medium.....	5.4	-----	-----	-----
Do.....	do.....	do.....	3.6	-----	-----	-----
Do.....	do.....	Not vigorous.....	3.0	-----	-----	-----
Do.....	do.....	do.....	2.3	-----	-----	-----

* Pollen from various pecan varieties when examined in 1929, 13 years after collection, showed the following percentages of defective grains: Frotscher, 4.5; Moneymaker, 6.2; Pan American, 9.8; President, 4.1; Teche, 30.6; Van Deman, 3.

TABLE 6.—Percentage of defective pollen as found by counting 1,000 grains in lactic acid mounts, 1928 and 1929—Continued

1929

Pecan variety	Location	Vigor	Average defective grains	Percentage of defective grains found at position indicated		
				Apex	Middle	Base
Moore.....	Experiment, Ga.....	Medium.....	6.3	9.8	2.5	6.7
Nelson.....	do.....	do.....	5.6	5.4	4.6	6.8
Do.....	Barnsville, Ga.....	do.....	12.3			
Pabst.....	Experiment, Ga.....	do.....	1.0	1.1	.9	.9
President.....	Tifton, Ga.....	do.....	6.5			
Robson.....	Experiment, Ga.....	do.....	2.7	2.2		3.2
San Saba.....	do.....	do.....	5.2	2.9	3.7	7.8
Schley.....	do.....	do.....	5.9	6.5	5.6	5.7
Do.....	Baconton, Ga.....	Vigorous.....	6.0			
Do.....	Griffin Ga.....	Not vigorous.....	37.5			
Do.....	Tifton, Ga.....	Vigorous.....	6.0			
Summers.....	do.....	do.....	16.4			
Stuart.....	Experiment, Ga.....	do.....	7.2			
Success.....	do.....	do.....	3.7			
Teche.....	do.....	do.....	1.2			
Van Deman.....	do.....	do.....	41.0	36.6	46.0	41.5
Do.....	do.....	do.....	32.1			
Do.....	Tifton, Ga.....	Not vigorous.....	40.0			
Do.....	Cordele, Ga.....	Vigorous.....	9.6			
Do.....	Baconton, Ga.....	do.....	26.0			
Wauchenhah.....	Experiment, Ga.....	Not vigorous.....	11.2			
Williams.....	Tifton, Ga.....	Vigorous.....	10.4			
		Fertilizer				
Stuart.....	Experiment, Ga.....	None.....	5.4			
Do.....	do.....	Complete.....	3.0			
Do.....	do.....	High P.....	5.6			
Do.....	do.....	High N.....	8.6			
Do.....	do.....	High K.....	7.2			
Do.....	do.....	Stable manure.....	6.5			

In 1928 pollen was much more plentiful in the orchards than in 1929, and the percentage of defective grains was about twice as high. The differences between the percentages of defective pollen shed from the apex, middle, and base of the same catkins is negligible. The Centennial and Mantura were the only varieties that produced pollen more than 19 per cent of which was defective in 1928 and 1929, and the Pabst was the only variety that produced pollen less than 5 per cent of which was defective in both years. The variation in percentages of defective pollen of the Mobile variety, taken from four localities and from trees of unequal vigor in 1929, is very small; also the variation in percentages of defective pollen taken from Stuart trees which received different kinds of fertilizers is insignificant. On the other hand, Schley pollen from nonvigorous trees at Griffin had about six times as high a percentage of defective pollen as that gathered at Experiment, Baconton, or Tifton, and Van Deman pollen from vigorous trees at Cordele had about one-third as high a percentage of defective pollen as that from trees of medium vigor at Baconton and about one-fourth as high as that from vigorous trees at Experiment or Tifton.

Table 7 shows the magnitude of variation of defective grains in successive lots of 100, that is, the percentage of defective grains in successive lots. The table includes 6 varieties and 12 lots of 1,000 grains each which were shown in Table 6. In general, it may be said that in counting 1,000 grains in lots of 100 grains each, the

lowest percentage of defective grains is about half that of the highest percentage.

TABLE 7.—*Magnitude of the variation (per cent) of defective grains as counted in successive lots of 100*

Percentage of defective grains at position mentioned in pollen from—									Percentage of defective grains in pollen from—		
Centennial			Jerome			Schley			Alley	Mantura	Curtis
Apex	Middle	Base	Apex	Middle	Base	Apex	Middle	Base			
34	32	30	33	33	44	11	20	13	30	57	54
30	34	32	19	35	38	13	14	21	16	72	51
35	30	33	36	47	42	9	16	15	16	80	52
35	34	44	24	37	40	15	15	12	13	57	53
32	38	41	40	33	45	10	22	9	17	74	54
33	40	44	30	35	43	16	17	8	19	74	77
43	29	42	36	46	47	10	13	16	25	68	60
34	36	34	31	34	54	15	17	13	21	77	56
45	34	33	40	39	39	17	14	18	17	28	56
19	38	38	35	30	30	12	12	14	21	81	48
33.5	34.5	37.1	32.4	37.1	42.2	12.8	16	14	19.5	71.2	56.1

The data recorded in Table 8 were obtained when the rubber-ring cells were used, as previously described.

TABLE 8.—*Pollen germination under varying conditions*

SERIES 1 *

Sugar used	Strength	Germination	Tube length	Grains bursting
	<i>Per cent</i>			
Maltose.....	20	0.5	Short.....	Few.
Do.....	15	.5	do.....	Do.
Do.....	10	1.0	do.....	Do.
Do.....	5	1.0	do.....	Do.
Sucrose.....	20	25.0	Long.....	None.
Do.....	15	30.0	do.....	Do.
Do.....	10	50.0	do.....	Do.
Do.....	5	40.0	do.....	Do.
Lactose.....	20	50.0	do.....	Do.
Do.....	15	40.0	do.....	Do.
Do.....	10	30.0	do.....	Do.
Do.....	5	30.0	do.....	Do.
Glucose.....	20	15.0	Short.....	Few.
Do.....	15	10.0	do.....	Do.
Do.....	10	5.0	do.....	Do.
Do.....	5	2.0	do.....	Do.
Fructose.....	20	0	do.....	Do.
Do.....	15	.5	do.....	Do.
Do.....	10	1.0	do.....	Do.
Do.....	5	1.0	do.....	Do.
Galactose.....	20	20.0	do.....	Do.
Do.....	15	15.0	do.....	Many.
Do.....	10	10.0	do.....	Do.
Do.....	5	5.0	do.....	Do.

* Using 2 per cent agar, pollen stored in laboratory for 24 hours, germination at 25° C., 4 drops of water in bottom of cell.

TABLE 8.—Pollen germination under varying conditions—Continued

SERIES 2^b

Reagent added	Amount used	Germination	Tube length	Grains bursting	Remarks
	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>	
Asparagine.....	1.0	75	Long.....	10	
Do.....	.5	80	do.....	10	
Do.....	.1	85	do.....	10	
Tannic acid.....	1.0	0	0	
Do.....	.5	0	0	
Do.....	.1	0	0	
Peptone.....	1.0	20	Long.....	10	
Do.....	.5	50	do.....	15	
Do.....	.1	75	do.....	20	
Sodium hydroxide.....	1.0	0	0	Stained brown.
Do.....	.5	0	0	Do.
Do.....	.1	0	0	Do.
Do.....	.05	20	Long.....	20	
Do.....	.025	30	do.....	20	
Check.....	75	do.....	10	

SERIES 3^c

Storage conditions	Storage period	Germination	Tube length	Grains bursting
	<i>Hours</i>	<i>Per cent</i>		
Fresh.....	70.0	Very long.....	None.
Laboratory.....	48	25.0	Medium.....	Do.
Desiccator.....	48	0	Do.
Moist chamber.....	48	30.0	Medium.....	Do.
25° C., dry.....	48	20.0	do.....	Do.
On ice.....	48	40.0	Short.....	Few.
Laboratory.....	72	15.0	do.....	Many.
Moist chamber.....	72	20.0	do.....	Do.
25° C., dry.....	72	0	None.
On ice.....	72	30.0	Short.....	Many.
Laboratory.....	96	.2	do.....	Few.
Moist chamber.....	96	10.0	do.....	Do.
On ice.....	96	20.0	do.....	Many.
Laboratory.....	120	0	None.
Moist chamber.....	120	5.0	Short.....	Do.
On ice.....	120	10.0	do.....	Many.
Moist chamber.....	144	0	None.
On ice.....	144	2.0	Short.....	Many.
In mail, moist.....	48	25.0	do.....	Do.
In mail, semimoist.....	48	20.0	do.....	Do.
In mail, dry.....	48	5.0	do.....	Do.
In mail, moist.....	192	5.0	do.....	Do.
In mail, semimoist.....	192	2.0	do.....	Do.
In mail, dry.....	192	0	do.....	Few.

^b Using 10 per cent sucrose, 1¼ per cent agar, Success pollen stored in laboratory for 24 hours, germination at 22° C., cell half filled with water.

^c Using 20 per cent sucrose, 2 per cent agar, germination at 25° C., 4 drops of water in cell.

TABLE 8.—Pollen germination under varying conditions—Continued

SERIES 4^a

Pecan variety	Period of storage	Percentage germination under storage conditions indicated									
		32° C.		23° C.		22° C.		In orchard		5° C.	
		Dry	Moist	Dry	Moist	Dry	Moist	Dry	Moist	Dry	Moist
	<i>Hours</i>										
Schley.....	24	0	0	0	65	0	65	30	64	50	65
Stuart.....	24	0	0	0	80	0	80	45	80	65	80
Van Deman.....	24	0	0	0	50	0	50	25	45	45	50
Schley.....	48	0	0	0	50	0	55	0	60	25	60
Stuart.....	48	0	0	0	65	0	70	0	75	35	75
Van Deman.....	48	0	0	0	30	0	40	0	45	20	45
Schley.....	72	-----	-----	-----	0	-----	20	-----	30	0	55
Stuart.....	72	-----	-----	-----	0	-----	25	-----	45	0	65
Van Deman.....	72	-----	-----	-----	0	-----	10	-----	20	0	40
Schley.....	96	-----	-----	-----	-----	-----	0	-----	15	-----	30
Stuart.....	96	-----	-----	-----	-----	-----	0	-----	25	-----	40
Van Deman.....	96	-----	-----	-----	-----	-----	0	-----	10	-----	25
Schley.....	120	-----	-----	-----	-----	-----	-----	-----	0	-----	3
Stuart.....	120	-----	-----	-----	-----	-----	-----	-----	0	-----	5
Van Deman.....	120	-----	-----	-----	-----	-----	-----	-----	0	-----	0
Beverage.....	120	0	0	0	0	0	0	0	0	0	0
Nelson.....	120	0	0	0	0	0	0	0	0	0	3
Robson.....	120	0	0	0	0	0	0	0	0	0	2
Centennial.....	120	0	0	0	0	0	0	0	0	0	0
Alley.....	120	0	0	0	0	0	0	0	0	0	1

SERIES 5^a

Germinating temperature	Percentage germination of—			Germinating temperature	Percentage germination of—		
	Schley	Stuart	Van Deman		Schley	Stuart	Van Deman
3° C.....	0	0	0	23° C.....	65	80	50
22° C.....	60	75	50	32° C.....	0	0	0

^a Using 20 per cent sucrose, 1½ per cent agar, germination at 23° C., cells half filled with water (percentage germination).

^b Using 10 per cent sucrose, 1½ per cent agar, cell half filled with water, fresh pollen.

Media containing 10 per cent sucrose, 1½ per cent agar, plus either one of the following formed a precipitate, failed to solidify at 20° C., and was not used: United States Pharmacopoeia citric acid, 1, 0.5, and 0.1 per cent; 91 per cent lactic acid, 0.5 and 0.1 per cent; United States Pharmacopoeia tannic acid, 1 per cent.

Under suitable conditions pollen began to germinate in 1½ hours and completed germination in 12 hours, but 14 to 18 hours were required for pollen tubes to reach full length before bursting. If allowed to remain longer than about 18 hours the tubes burst and emptied their contents into the surrounding medium. (Fig. 24.)

Bursting of pollen tubes is distinct from "pseudo germination" or bursting of ungerminated grains as described by Andronesco (1) and as used in Table 8. If the conditions remain favorable for growth all of the pollen tubes will burst in about 30 hours.

No tubes in artificial media have been found to branch, as occurs in the tube growth in the pistils (31); nor has any of the germination percentages equaled the percentage of normal pollen as shown by counts in lactic acid.

Pollen failed to germinate in pure water, or in any concentration of agar which did not solidify at 25° C. Satisfactory results were obtained with 1½ or 2 per cent agar when sugar was added. No germination was obtained when no water was placed in the cell to provide moisture for the pollen. From four to eight drops of water were found best for this; less than this amount did not keep the pollen sufficiently moist for germination, and much more than this caused excessive bursting of grains.

Data in Table 8 show that of the six sugars used in the media for germinating pollen, lactose and sucrose gave highest percentage

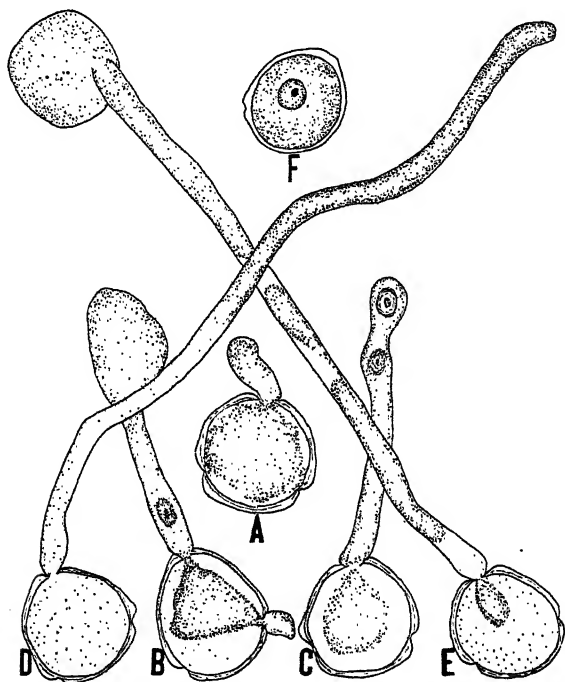


FIGURE 24.—Camera lucida drawing of stages in the germination of pollen grains on sugar-agar medium: A, Normal germination; B, formation of two tubes from a single pollen grain; B and E, swelling at end of tube which may later burst; C, two nuclei in the pollen tube; D, a normal tube; F, section of a pollen grain 10 days before shedding. $\times 750$

germination and least bursting of the grains; galactose and glucose produced fair germination; and fructose and maltose caused an extremely low percentage germination and a high percentage of bursting.

One-tenth of 1 or one-half of 1 per cent asparagine when added to the medium produced a slight increase in percentage of germination; 1 per cent neither increased nor decreased the percentage of germination. More than 0.1 per cent of peptone caused a decrease in percentage of germination. Tannic acid prevented germination when 1, 0.5, or 0.1 per cent was added to the medium. Sodium hydroxide decreased germination when as low as 0.025 per cent was added to the medium, and more than 0.1 per cent prevented germination.

TABLE 9.—*Relation between the size of crop of nuts of one year and the production of catkins and pistillate flowers the following year—Continued*

Pecan variety	Age of tree	1928 crop of nuts	1928 crop rating	Rating of 1929 crop of—	
				Catkins	Pistillate flowers
	<i>Years</i>				
Mobile	18	63 pounds	A	E	F
Do.	18		F	A	A
Do.	18		F	A	A
Do.	18		F	A	A
Do.	18		F	A	A
Do.	18		F	A	A
Do.	18		F	A	A
Do.	18		F	A	A
Do.	18	3,202 nuts ^a	F	A	A
Do.	18	2,938 nuts ^a	A	A	D
Do.	18	4,704 nuts ^a	A	A	C
Do.	18	856 nuts	D	A	A
Do.	25	22,735 nuts ^a	A	F	F
Do.	22	15 pounds	D	B	B
Do.	11	41 pounds	A	F	F
Do.	11	54 pounds	A	F	F
Do.	11	28 pounds	A	F	F
Do.	11	29 pounds	A	F	F
Do.	11	25 pounds	A	F	F
Do.	11	33 pounds	A	F	F
Do.	11	43 pounds	A	F	F
Moneymaker	20	46 pounds	C	F	F
Moore	12	21 pounds	B	A	A
Do.	12	6 pounds	E	A	A
Nelson	20	110 pounds ^a	A	B	F
Do.	18	66 pounds ^a	A	B	F
Do.	19		A	E	B
Do.	19		A	E	F
Do.	19		A	E	F
Do.	19		A	E	F
Do.	19		A	E	F
Do.	19		A	E	F
Do.	19		A	E	F
Do.	19		A	E	F
Do.	19		A	E	F
Do.	19		A	E	F
Do.	19		A	E	F
Do.	16	1,504 nuts ^a	A	A	B
Do.	16	1,354 nuts ^a	A	A	B
Do.	16	1,779 nuts ^a	A	A	C
Pabst	20	91 pounds	A	B	F
Randal	20	96 pounds	A	F	F
Robson	20	48 pounds	C	B	D
Rome	12	11 pounds	C	B	B
Do.	20	40 pounds	C	B	B
San Saba	18	25 pounds	C	B	B
Schley	12	55 pounds	A	E	E
Do.	18		A	C	A
Do.	18		A	C	A
Do.	8		A	C	C
Do.	8	17 pounds	B	C	E
Do.	9	22 pounds	B	C	A
Do.	18		A	C	A
Do.	18		A	B	A
Do.	18		A	B	B
Do.	18		A	B	B
Do.	18		A	B	B
Do.	22		D	B	B
Do.	22		D	B	B
Do.	22		D	B	B
Do.	22		D	C	B
Do.	22		E	C	B
Do.	16	615 nuts	B	D	B
Do.	18	689 nuts	C	D	B
Stuart	20	2 pounds	E	A	B
Do.	18	2,161 nuts	C	B	B
Do.	18	1,961 nuts	C	B	B
Do.	18	999 nuts	D	B	B
Do.	18	2,136 nuts	C	B	B
Do.	18	1,273 nuts	C	B	B
Do.	18	992 nuts	D	B	B
Do.	18	1,533 nuts	C	B	B
Do.	16	852 nuts	C	C	B

^a Nuts poorly filled.

TABLE 9.—*Relation between the size of crop of nuts of one year and the production of catkins and pistillate flowers the following year—Continued*

Pecan variety	Age of tree	1928 crop of nuts	1928 crop rating	Rating of 1929 crop of—	
				Catkins	Pistillate flowers
Stuart	Years				
Do.	16	717 nuts	C	A	B
Do.	16	164 nuts	D	A	A
Do.	18	763 nuts	D	A	A
Do.	18	607 nuts	D	A	A
Do.	18	625 nuts	D	A	A
Do.	18	663 nuts	D	B	A
Do.	11	3 pounds	E	A	B
Do.	11	2 pounds	E	A	B
Do.	11	7 pounds	F	A	B
Do.	11	1 pound	E	A	B
Do.	11	4 pounds	E	A	B
Do.	11	17 pounds	C	A	B
Do.	11	1 pound	E	A	B
Do.	11	4 pounds	E	A	B
Do.	11	3 pounds	E	A	B
Do.	11	7 pounds	E	A	B
Do.	11	16 pounds	C	A	B
Do.	11	3 pounds	E	A	B
Do.	11	13 pounds	D	A	B
Do.	11	9 pounds	D	A	B
Do.	11	12 pounds	D	A	B
Do.	11	17 pounds	C	A	B
Do.	11	17 pounds	C	A	R
Do.	11	6 pounds	E	A	B
Do.	11	9 pounds	D	A	B
Do.	11	1 pound	E	A	B
Do.	11	16 pounds	C	A	B
Do.	11	9 pounds	D	A	B
Do.	11	9 pounds	D	A	B
Do.	11	6 pounds	E	A	B
Do.	11	6 pounds	E	A	B
Do.	11	17 pounds	C	A	B
Do.	11	6 pounds	E	A	B
Do.	11	8 pounds	D	A	B
Do.	11	11 pounds	D	A	B
Do.	11	16 pounds	C	A	B
Do.	11	8 pounds	D	A	B
Do.	11	2 pounds	E	A	B
Success	12	4 pounds	E	A	A
Teche	20	55 pounds	B	D	D
Do.	20	81 pounds	A	F	F
Do.	20	61 pounds	B	D	F
Do.	20	71 pounds	A	F	F
Do.	18	268 nuts	E	B	A
Do.	18	1,023 nuts	C	B	A
Do.	18	1,167 nuts	C	B	A
Do.	18	434 nuts	D	F	A
Do.	11	24 pounds	B	D	C
Do.	11	21 pounds	B	F	C
Do.	11	18 pounds	B	E	C
Do.	11	8 pounds	C	D	D
Do.	11	18 pounds	B	E	F
Do.	11	15 pounds	C	D	F
Do.	11	7 pounds	B	B	F
Do.	11	24 pounds	C	C	F
Do.	11	20 pounds	A	A	F
Van Deman	20	19 pounds	C	A	F
Do.	19		C	A	C
Do.	19		C	A	C
Do.	19		C	A	C
Do.	19		C	A	C
Do.	19		C	A	C
Do.	19		E	A	A
Wilson	12		C	A	C
Do.	12		C	A	C

TABLE 9.—*Relation between the size of crop of nuts of one year and the production of catkins and pistillate flowers the following year*—Continued

Pecan variety	Age of tree	1926 crop rating	Rating of 1927 crop of—		Pecan variety	Age of tree	1926 crop rating	Rating of 1927 crop of—	
			Catkins	Pistillate flowers				Catkins	Pistillate flowers
Mobile.....	Years 9	A	F	F	Stuart.....	Years 9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Do.....	9	A	F	F	Do.....	9	C	A	A
Stuart.....	9	C	A	A	Do.....	9	C	A	A
Do.....	9	C	A	A	Do.....	9	C	A	A
Do.....	9	C	A	A	Do.....	9	C	A	F
Do.....	9	C	A	C	Do.....	9	C	A	B
Do.....	9	C	A	A	Do.....	9	C	A	B
Do.....	9	C	A	A	Do.....	9	C	A	F
Do.....	9	C	A	A	Do.....	9	C	A	B
Do.....	9	C	A	A	Do.....	9	C	A	C
Do.....	9	C	A	A	Do.....	9	C	A	C
Do.....	9	C	A	A	Do.....	9	C	A	C
Do.....	9	C	A	A	Do.....	9	C	A	F

SUMMARY OF ALL VARIETIES

Previous year's crop of nuts	Current year's crop of—											
	Catkins						Pistillate flowers					
	A	B	C	D	E	F	A	B	C	D	E	F
56 A's.....	5	6	7	0	14	24	10	7	1	1	2	35
27 B's.....	2	0	15	5	2	3	2	14	4	1	2	4
71 C's.....	52	13	2	3	0	1	24	23	11	5	0	8
31 D's.....	16	14	1	0	0	0	12	19	0	0	0	0
29 E's.....	26	2	1	0	0	0	9	19	1	0	0	0
6 F's.....	6	0	0	0	0	0	6	0	0	0	0	0

DISCUSSION

The general course of development of the pollen of the pecan is the same as that of other fruits. The fact that floral differentiation occurs a year before the pollen sheds shows that either the quantity or the quality of pollen may be influenced by the condition of the trees during the summer, fall, or winter previous to the shedding of pollen. On the other hand, pistillate flower differentiation occurs only about four months before the flowers become receptive. Therefore it would be expected that the condition of the tree during the previous year would influence staminate flower development much more than it would pistillate flower development.

Irregularities and abnormalities were found in the development of pollen in both groups of varieties. In a few cases there were rather high percentages of defective pollen. However, in view of the rather universal abundance of catkin formation, and therefore of pollen, it seems unlikely that pollen defects or impotence are factors of im-

portance in limiting the setting of fruit, except in cases of an isolated tree or variety, and then only when conditions are such as to cause a failure of staminate flowers to develop.

On the other hand, the more or less complete dichogamy that characterizes the pecan may occasion pollination difficulties. This is accentuated by the fact that pollen shedding is practically inhibited when the humidity remains above a certain point for any considerable period. It is still further accentuated by the fact that the period of receptivity of the stigmas is greatly reduced if the weather is very dry. In selecting varieties, therefore, the question of securing proper pollination should be considered. For practical purposes it seems that at least two varieties of each group should be included in a commercial planting. This would provide for at least one variety to shed pollen early in the season and at least one variety to shed late.

It also seems that the number of trees of either of these two varieties could be reduced to one-twentieth of the total number of trees.

SUMMARY AND CONCLUSION

Catkin primordia are differentiated in lateral buds on new shoots throughout the growing season. In varieties of Group 1, anthers are differentiated in the fall of the year in which the catkin primordia are formed; in varieties of Group 2 anthers are differentiated early the following spring.

No abnormal behavior was observed in the development of catkin primordia, individual flower primordia, archesporial-cell stage or the mother-cell stage, but abnormalities occurred in all varieties studied during the reduction-division stage.

The smear method for counting the number of tetrads per anther, and the lactic acid method for determining the percentage of defective grains of pollen, have been successfully applied to pecan pollen studies. A method for quantitatively catching pollen from the air at various distances from pollen-shedding trees and at various heights from the ground was developed.

Though an entirely satisfactory method for germinating pecan pollen has not been developed, much was learned about the temperature, humidity, and nutritional requirements for germination on artificial media. The longevity of pollen was found to be about equal to or less than the period of receptivity of the stigmas, which indicates that there must be a continuous shedding of pollen throughout the period of receptivity of the stigmas.

When 1,000 grains from each of 169 lots of pollen were examined by the lactic acid method, the defective grains were found to range from 0 to 81.7 per cent. Pollen produced on trees of low vigor or on trees which bore very heavy crops of catkins had a slightly higher percentage of defective grains than pollen produced on trees of high vigor or on trees which had a light crop of catkins.

Records of blooming dates showed that either homogamy, protandry, or protogyny may occur in pecans.

It was found that pollen sheds only when the relative humidity of the air is below about 85 per cent and that practically all shedding occurs between 9 a. m. and sundown.

Conditions which were optimum for pollen shedding were destructive to the vitality of pollen. Optimum conditions for shedding of pollen are those which exist in the orchard on a warm, sunny day;

and optimum conditions for germination are found in an orchard on a warm, dewy night.

From the data contained herein it seems that all of the methods of studying pecans which involve hand pollination, used by the writer and others, have been somewhat at fault in that account was not taken of the fact that during times of low humidity there may be an enormous amount of pollen in the air even at great distances from pollen-shedding trees.

The size of the crop of nuts of one year was found to influence the size of crop of both staminate and pistillate flowers of the following year. A very heavy crop of nuts usually follows and is followed by a lighter crop. Certain varieties of pecan produce a medium-size crop of staminate flowers, pistillate flowers, and nuts each year.

LITERATURE CITED

- (1) ANDRONESCU, D. I.
1915. THE PHYSIOLOGY OF ZEA MAYS WITH SPECIAL REFERENCE TO VITALITY. 36 p., illus. (Thesis, University of Illinois, Urbana).
Publ. by Dept. Agr., Kingdom of Roumania.
- (2) ANTHONY, S., and HARLAN, H. V.
1920. GERMINATION OF BARLEY POLLEN. Jour. Agr. Research 18: 525-536, illus.
- (3) ASAMI, Y.
1927. POLLEN ABORTION IN THE SHANGHAI PEACH. Jour. Sci. Agr. Soc. Japan 297: 364-372, illus. [English abstract Japan. Jour. Bot. 4: (1). 1928.]
- (4) AUCHTER, E. C.
1922. APPLE POLLEN AND POLLINATION STUDIES IN MARYLAND. Amer. Soc. Hort. Sci. Proc. (1921) 18: 51-80.
- (5) BEAUMONT, J. H., and KNIGHT, L. I.
1923. APPLE POLLEN GERMINATION STUDIES. Amer. Soc. Hort. Sci. Proc. (1922) 19: 151-163.
- (6) BOOTH, N. O.
1908. SOME PHASES OF POLLINATION. Amer. Soc. Hort. Sci. Proc. (1906) 4: 20-26.
- (7) COULTER, J. M., and CHAMBERLAIN, C. J.
1915. MORPHOLOGY OF ANGIOSPERMS. Illus. D. Appleton & Co., New York.
- (8) DORSEY, M. J.
1919. A STUDY OF STERILITY IN THE PLUM. Genetics 4: 417-488, illus.
- (9) FLORIN, R.
1927. POLLEN PRODUCTION AND INCOMPATIBILITIES IN APPLES AND PEARS. Mem. Hort. Soc. N. Y. 3: 87-118, illus.
- (10) HIGGINS, B. B.
1917. A DISEASE OF PECAN CATKINS. Phytopathology 7: 42-46, illus.
- (11) HOWLETT, F. S.
1927. APPLE POLLINATION STUDIES IN OHIO. Ohio Agr. Expt. Sta. Bul. 404, 84 p., illus.
- (12) ISBELL, C. L.
1928. GROWTH STUDIES OF THE PECAN. Ala. Agr. Expt. Sta. Bul. 226, 68 p., illus.
- (13) KAUFMANN, B. P.
1927. THE VALUE OF THE SMEAR METHOD FOR PLANT CYTOLOGY. Stain Technol. 2: 88-90.
- (14) KNIGHT, L. I.
1918. PHYSIOLOGICAL ASPECTS OF SELF-STERILITY OF THE APPLE. Amer. Soc. Hort. Sci. Proc. (1917) 14: 101-105.
- (15) KNOWLTON, H. E.
1922. STUDIES IN POLLEN WITH SPECIAL REFERENCE TO LONGEVITY. N. Y. Cornell Agr. Expt. Sta. Mem. 52, p. 751-793.
- (16) ———
[1925]. POLLEN ABORTION IN THE PEACH. Amer. Soc. Hort. Sci. Proc. (1924) 21: 67-69.

- (17) LIDFORSS, B.
1896. ZUR BIOLOGIE DES POLLENS. *Jahrb. Wiss. Bot.* 29: 1-38, illus.
- (18) SCHUSTER, C. E.
1924. FILBERTS. PART I. GROWING FILBERTS IN OREGON. PART II. EXPERIMENTAL DATA ON FILBERT POLLINATION. *Oreg. Agr. Expt. Sta. Bul.* 208, 39 p., illus.
- (19) SHOEMAKER, J. S.
1926. POLLEN DEVELOPMENT IN THE APPLE, WITH SPECIAL REFERENCE TO CHROMOSOME BEHAVIOR. *Bot. Gaz.* 81: 148-172, illus.
- (20) ———
1928. CHERRY POLLINATION STUDIES. *Ohio Agr. Expt. Sta. Bul.* 422, 34 p., illus.
- (21) SHUHART, D. V.
1927. THE MORPHOLOGICAL DIFFERENTIATION OF PISTILLATE FLOWERS OF THE PECAN. *Jour. Agr. Research* 34: 687-696, illus.
- (22) SNOW, R.
1925. GERMINATION TESTS WITH POLLEN OF STOCKS. *Jour. Genetics* 15: 237-243.
- (23) STUCKEY, H. P.
1916. THE TWO GROUPS OF VARIETIES OF THE HICORIA PECAN AND THEIR RELATION TO SELF-STERILITY. *Ga. Agr. Expt. Sta. Bul.* 124, p. 125-148, illus.
- (24) VALLEAU, W. D.
1918. STERILITY IN THE STRAWBERRY. *Jour. Agr. Research* 12: 613-670, illus.
- (25) WAUGH, F. A.
1901. REPORT OF THE HORTICULTURIST. *Vt. Agr. Expt. Sta. Ann. Rpt.* (1899/1900) 13: 333-373, illus.
- (26) WOODROOF, J. G.
1924. THE DEVELOPMENT OF PECAN BUDS AND THE QUANTITATIVE PRODUCTION OF POLLEN. *Ga. Agr. Expt. Sta. Bul.* 144, p. 134-161, illus.
- (27) ———
1926. THE FRUIT-BUD, THE FLOWER, AND THEN THE PECAN NUT. *Natl. Pecan Growers' Assoc. Proc.* 25: 81-92, illus.
- (28) ——— and WOODROOF, N. C.
1926. FRUIT-BUD DIFFERENTIATION AND SUBSEQUENT DEVELOPMENT OF FLOWERS IN HICORIA PECAN. *Jour. Agr. Research* 33: 677-685, illus.
- (29) ——— and WOODROOF, N. C.
1927. THE DEVELOPMENT OF THE PECAN NUT (HICORIA PECAN) FROM FLOWER TO MATURITY. *Jour. Agr. Research* 34: 1049-1063, illus.
- (30) ——— WOODROOF, N. C., and BAILEY, J. E.
1928. UNFRUITFULNESS OF THE PECAN. *Ga. Agr. Expt. Sta. Bul.* 149, 40 p., illus.
- (31) WOODROOF, N. C.
1928. DEVELOPMENT OF THE EMBRYO SAC AND YOUNG EMBRYO IN HICORIA PECAN. *Amer. Jour. Bot.* 15: 416-521, illus.

INHERITANCE OF ANTHOCYAN PIGMENTATION IN RICE¹

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INTRODUCTION

The organs of the principal rice varieties commercially grown in the United States are uncolored (green) in the vegetative condition. There are other rice varieties, however, in which one or more of the vegetative organs are colored. The colors present in these organs usually are purples and reds of varying shades and intensity.

The colors in the vegetative organs of rice are due to the presence of anthocyan pigments, although other pigments may be present. The anthocyan pigments, which are soluble in the cell sap, often mask to a large extent the appearance of chlorophyll in the different organs. The inheritance of colors due to anthocyan pigments has been studied in maize, stock, snapdragon, rice, and other species, and it has been found to be in accordance with Mendel's laws.

The studies reported in this paper were undertaken to determine the mode of inheritance of anthocyan color in crosses between rice varieties having white kernels and normally green vegetative organs and those having kernels red when hulled and certain of the vegetative organs colored.

In this paper the word "green" denotes normal green color, except in the case of awns, which are normally straw-colored at maturity, while the word "colored" or the name of the color is used to denote the presence of anthocyan pigmentation.

TERMINOLOGY OF GLUMES AND THEIR PARTS³

In many papers discussing rice varieties, the terminology of the various glumes is confusing. The two small glumes at the base of the spikelet are referred to variously as outer glumes, lower glumes, empty glumes, sterile glumes, or glumes 1 and 2. The lemma and palea, which inclose the flower and subsequently the kernel, are called variously the inner glumes, upper glumes, floral glumes, and glumes 3 and 4. In conformity with accepted American usage, the first pair, or outer glumes, will be designated simply as glumes, and the two floral glumes as lemma and palea, respectively.

The short excurrent tips of the major fibrovascular bundles of the floral glumes, of which the lemma bears usually one but sometimes

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³ The writer is indebted to C. R. Ball for assistance in working out a satisfactory terminology for these organs.

three, and the palea one, have been referred to by many writers on oriental rices as the "apiculus of the glumes."

In rice varieties having the vascular apiculi colored, the pigment frequently extends to the immediately adjacent or subtending parenchymatous tissue at the apex of the lemma and the palea. The phrases "glume tips" and "glume apexes" have been used by writers abroad to designate either or both of these tissues. In the present paper these combined tissues, "glume tips" and "glume apexes," are called lemma and palea apexes.

MATERIAL AND METHODS

The five rice varieties used as female parents were Niro Vialone, Butte (C. I.⁴ 1564), Colusa (C. I. 1600), Eureka, and an unnamed variety (C. I. 5346). These are all of the Japanese type. Niro Vialone is an early maturing Italian variety. Many of its vegetative organs are purplish in color. Normally it is awnless, but occasionally a purplish mucro, or awnlet, varying in length from 1 to 2 mm., may be observed. The lemma and palea apexes, the (outer) glumes, the stigmas, the pulvini, the bases of the ligules, the auricles, the leaf sheaths, the leaf blades, the nodes, and the internodes are all purplish in color. Butte is a midseason awned variety with only the awns and the apiculi reddish in color. Colusa is an early maturing awnless variety normally without any colored organs. Eureka is an early maturing awnless variety which has no color except in the apiculi, which are red. The unnamed variety (C. I. 5346) is an early maturing, partly awned variety, without any anthocyan color.

The four varieties used as male parents were Caloro, Colusa, Italian Red, and Wataribune. Caloro is a midseason, partly awned variety. Normally about one-half of the spikelets on a panicle are awnless. All the organs, including the awns, are without anthocyan color. Wataribune is a late maturing, partly awned variety, without colored organs. The red variety used as the male parent in two crosses is locally known as Italian Red because it is supposed to have been introduced into California with seed rice which came directly or indirectly from Italy. Italian Red is an early maturing, partly awned variety, the seeds of which shatter quite readily. It has purple awns, purplish leaf margins and leaf sheaths, and reddish-purple ligules, auricles, and pulvini. The nodes are dark purple, and the internodes near the nodes show a tinge of purple. The glumes, lemma and palea apexes, and stigmas are purplish, and the hulled kernels are red.

The cross Niro Vialone×Caloro was made by the writer at the Biggs Rice Field Station in the fall of 1922; the crosses Butte×Colusa and Eureka×Caloro in the fall of 1923; and the crosses Colusa×Italian Red, C. I. 5346×Italian Red, and Niro Vialone×Wataribune in the fall of 1924.

The hybrid seeds obtained were sown in paper pots, one seed in each pot. The pots were irrigated as frequently as necessary to maintain suitable moisture conditions for germination and growth. After the seedlings had emerged the bottoms of the pots were removed and the seedlings were transplanted to the nursery. The plants were spaced from 8 to 12 inches apart in rows 2 feet apart. The rice was

⁴ C. I. refers to accession number of the Office of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

irrigated at frequent intervals after transplanting until the land was continuously submerged.

The F_2 and F_3 progenies were grown from seeds sown in the field nursery. The plants were spaced from 6 to 12 inches apart in rows 2 feet apart.

ASSUMPTION OF A THREE-FACTOR HYPOTHESIS

In the study of the color characters reported in the cross Niro Vialone \times Caloro it is assumed that the factor C must be present in order that anthocyan color may develop in any of the organs. When the factor P is present with C the color of certain organs is purple or purple striped, but when P is absent and C is present their color is red. It is also assumed that the factor A is necessary for the full expression of the purple color in certain organs.

The colors in the Niro Vialone variety, therefore, are assumed to be due to the interaction of the three dominant complementary factors ACP . The Caloro variety possesses no anthocyan pigments and is assigned the factorial composition acp . The F_1 hybrids, therefore, would have the factorial constitution $AaCcPp$ and would be purple. The selfed F_1 plants would produce F_2 progeny falling into eight phenotypic classes, of the genotypes and in the ratios shown in Table 1. For convenience in discussing the inheritance of color the phenotypes are numbered from 1 to 8, inclusive. (Table 2.)

TABLE 1.—*Genotypic classes resulting in F_2 from the cross Niro Vialone ($AACCP$) \times Caloro (acp)*

Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8
1 $AACCP$	1 $AACcP$	1 $AaCCP$	1 $aaCCP$	1 $AaCcP$	1 $aaCCp$	1 $aaccP$	1 $aaccp$
2 $AACCP$	2 $AACcP$	2 $AaCCp$	2 $aaCCp$	2 $AaCcP$	2 $aaCCp$	2 $aaccP$	2 $aaccp$
2 $AaCCP$	2 $AaCCp$	2 $AaccP$	2 $aaCcP$	2 $AaCcP$	2 $aaCcP$	2 $aaccP$	2 $aaccp$
4 $AaCCP$	4 $AaCcP$	4 $AaccP$	4 $aaCcP$	4 $AaCcP$	4 $aaCcP$	4 $aaccP$	4 $aaccp$
4 $AaCCp$	4 $AaCCp$	4 $Aaccp$	4 $aaCcP$	4 $AaCcP$	4 $aaCcP$	4 $aaccP$	4 $aaccp$
4 $AaCCp$	4 $AaCCp$	4 $Aaccp$	4 $aaCcP$	4 $AaCcP$	4 $aaCcP$	4 $aaccP$	4 $aaccp$
8 $AaCCp$	8 $AaCCp$	8 $Aaccp$	8 $aaCcP$	8 $AaCcP$	8 $aaCcP$	8 $aaccP$	8 $aaccp$
27 ACP	9 ACp	9 AcP	9 aCP	3 Acp	3 aCp	3 acP	1 acp

TABLE 2.—*Phenotypic classes present in the F_2 progeny from the cross Niro Vialone ($AACCP$) \times Caloro (acp)*

Class	Phenotypes	Color of organs
1	ACP	All organs under consideration, purple.
2	ACp	Awns and lemma and palea apices, red; other organs, green.
3	AcP	All organs green.
4	aCP	Leaves and leaf sheaths, purple striped; awns, lemma and palea apices, internodes, glumes, and stigmas, purple; all other organs, green.
5	Acp	All organs green.
6	aCp	Awns and lemma and palea apices, red; other organs, green.
7	acP	All organs green.
8	acp	Do.

The inheritance of the color in the cross Niro Vialone \times Caloro, using the 3-factor hypothesis as a basis for analysis, with data from other crosses, is presented in the following pages.

The awns that were present on the F_1 plants were purplish in color. Most of the spikelets were awnless, however. The leaves,

leaf sheaths, apexes, internodes, glumes, stigmas, ligules, auricles, pulvini, and nodes were purplish in color, but of a lighter purple than those of the Niro Vialone parent. Purple color, therefore, was dominant to green in F_1 .

Four separate linkage groups for color inheritance were obtained in F_2 , namely: (1) Colored leaves and leaf sheaths; (2) colored awns and lemma and palea apexes; (3) colored internodes, glumes, and stigmas; and (4) colored nodes, ligules, auricles, and pulvini.

The theoretical ratios in F_2 for plants within each of the color-linkage groups and the phenotypic classes of which they are made up are as follows:

Color of leaves and leaf sheaths.—Ratio 27 purple, phenotypic class 1; 9 purple striped, phenotypic class 4; 28 green, phenotypic classes 2, 3, 5, 6, 7, and 8.

Color of awns and lemma and palea apexes.—Ratio 9 purple, phenotypic classes 1 and 4; 3 red, phenotypic classes 2 and 6; 4 green, phenotypic classes 3, 5, 7, and 8.

Color of internodes, glumes, and stigmas.—Ratio 9 purple, phenotypic classes 1 and 4; 7 green, phenotypic classes 2, 3, 5, 6, 7, and 8.

Color of nodes, ligules, auricles, and pulvini.—Ratio 27 purple, phenotypic class 1; 37 green, phenotypic classes 2 to 8.

COLOR OF LEAVES AND LEAF SHEATHS

The normal color of the leaves and leaf sheaths in most rice varieties of the Japanese type is some shade of green. In certain varieties, however, the green in the leaves and leaf sheaths is partially masked by soluble anthocyan pigments, which are red, purple, or purplish black. The color in the leaves ordinarily is most pronounced at the base and along the margins, whereas that in the leaf sheaths usually is most pronounced along the midribs.

IN NIRO VIALONE \times CALORO

The inheritance of color in the leaves and leaf sheaths was studied in a cross between Niro Vialone, an Italian variety having purplish leaves and leaf sheaths, and Caloro, having green leaves and leaf sheaths.

In this cross the leaves and leaf sheaths of the F_1 hybrid were purple, of a somewhat lighter shade than those of the female parent.

F_2 PROGENY

The segregation in F_2 for color of leaves and leaf sheaths is shown in Table 3. The data do not agree well with the expected 27:9:28 ratio. The probability that the deviations observed in this ratio are due to random sampling is only about 1 in 80, which indicates that some disturbing forces may be affecting the results in this case. These disturbing forces may be a fourth factor.

TABLE 3.—Segregation of 207 F_2 hybrids from the cross Niro Vialone \times Caloro for color of leaves and leaf sheaths, at Biggs, Calif., 1924

Color of leaves and leaf sheaths	Number of plants—		Deviation
	Observed	Expected in 27:9:28 ratio *	
Purple.....	100	87.33	+12.67
Purple striped.....	15	29.11	-14.11
Green.....	92	90.56	+1.44

* $\chi^2=8.7004$. $P=0.0133$.

The plants with purple-striped leaves and leaf sheaths had green nodes, ligules, auricles, and pulvini, while in the purple-leaved group these organs were purple. The two groups, therefore, were very easily classified. The F_2 population was small; but, with a much larger population in the F_3 generation, the deviations from the expected 27:9:28 ratio are large, and the value for P shows a very poor fit. (See Table 5.)

If, on the assumption of a 3-factor hypothesis, the F_2 population is placed in four phenotypic groups, the data shown in Table 4 are obtained.

TABLE 4.—Classification in four phenotypic groups of the F_2 population of the cross *Niro Vialone* \times *Caloro* on the basis of a 3-factor hypothesis

Phenotypes	Ratio	Observed	Calculated	$\frac{(o-c)^2}{c}$
All organs purple.....	27	100	87.33	1.8382
Leaves and leaf sheaths purple striped.....	9	15	29.11	6.8393
Awns and lemma and palea apices red.....	12	30	38.81	2.0000
All organs green.....	16	62	51.75	2.0301
	64	207	207.00	$\chi=12.7076$

The value for P in this case is 0.0054, which indicates a poor fit.

An inspection of the values of $\frac{(o-c)^2}{c}$ show that the second phenotypic group, plants having purple-striped leaves and leaf sheaths, are materially at variance, whereas the other groups do not vary markedly from the observed ratio. This indicates that the unknown disturbing forces are probably located in the purple-striped group.

The purple-striped group (*aCP*) differs from the purple group (*ACP*) for the factor *A*. By grouping the F_2 plants on the theoretical genotypic basis, the *C* factor is present in 145 colored plants and absent in 62 green plants.

These numbers agree well with a 3:1 ratio. The deviation is 10.25 plants with a probable error of ± 4.20 . The *C* factor appears, therefore, to segregate independently. The *P* factor is present in 161 F_2 plants and absent in 46. These numbers also agree with a 3:1 ratio. The deviation is 5.75 plants with a probable error of ± 4.20 . The *P* factor appears to segregate independently. The *A* factor is present in 100 F_2 plants and absent in 15 plants having purple-striped leaves and leaf sheaths. These numbers do not agree well with a 3:1 ratio. The deviation is 13.75 plants with a probable error of ± 3.13 . The deviation is more than four times the probable error, which indicates that some disturbing forces are affecting the results in this case. These forces may be a fourth factor. However, the segregations in F_2 and F_3 for all characters studied are in general agreement with the 3-factor hypothesis, except for the color of leaves and leaf sheaths.

Takezaki (16)⁵ found that purple leaves were dominant to green in F_1 rice hybrids, and that segregation in F_2 was in the ratio of 27 plants with purple leaves to 37 with green. The correctness of this

⁵ Reference is made by number (italic) to "Literature cited," p. 1127.

ratio was verified by growing F_3 progeny. This ratio indicates that, for the varieties used, the purple color was due to the interaction of three dominant genetic factors.

Hector (2, 3), in studies on the inheritance of color in the leaf sheaths of rice, found that color was dominant to lack of color in F_1 . In F_2 he obtained ratios of 3:1, 9:7, 15:1, and 27:37 for plants with colored and green leaf sheaths, respectively. Parnell and others (13), Roy (15), and Nagai (12) found that segregation in F_2 was in the ratio of 3 plants with colored leaf sheaths to 1 with green. The colored variety used by Roy (15) had striped leaf sheaths. Roy also reports one segregation that produced 9 plants with colored leaf sheaths to 7 with green. These results indicate that in some varieties of rice a single genetic factor is responsible for the color of the leaf sheaths, while in other varieties the development of color apparently is due to the interaction of two or three dominant complementary factors.

The deficiency in the purple-striped group is large but probably not large enough to warrant a rejection of the 3-factor hypothesis. It is possible that the seeds of the purple-striped plants do not germinate so well as those of the other two groups, or, if they do germinate as well, the seedlings may not be so hardy as those of plants with purple leaves. The death of the less hardy purple-striped seedlings would result in a deficiency in this group. There is no evidence that this has occurred here, but other experiments at Biggs have shown that under field conditions only one-half to two-thirds of the seeds sown produce seedlings, and many of these fail to reach maturity. Therefore, if there are differences in the hardiness of these three groups of plants, these differences could lead to a deficiency in one group and an apparent excess of plants in either or both of the other groups.

F_3 PROGENY

To check the results obtained with the F_2 progeny and the 3-factor hypothesis indicated by them, F_3 populations were grown in 1925.

In all, 47 F_3 families were grown, 18 of which were from the purple group (phenotypic class 1), 9 from the purple-striped group (phenotypic class 4), and 20 from the green group (phenotypic classes 2, 3, 5, 6, 7, and 8). The 20 F_3 families from the green plants, consisting of 2,153 plants, all bred true for green leaves and leaf sheaths as was expected. The segregation of the F_3 progenies is shown in Table 5. Of the 18 F_3 families from the group having purple leaves and leaf sheaths, 4 families, consisting of 483 plants, bred true for purple leaves and leaf sheaths; 1 family segregated in the ratio of approximately 3 plants with purple leaves and leaf sheaths to 1 with these organs green; 1 family segregated in the ratio of 9 plants with purple leaves and leaf sheaths to 7 with green; and the remaining 12 families segregated into three groups similar to those obtained in F_2 .

The large plus deviation for the purple group and the marked minus deviations for the purple-striped and green groups are the outstanding inconsistencies noted in Table 5. These large deviations no doubt are significant. Whether they are due, as has been suggested, to a fourth factor or a difference in the germination of the seed of each group or to a difference in hardiness which results in a higher mortality of the purple-striped and green seedlings has not been determined. The results do show, however, that some factor or factors, genetic or external, affected the results in this case.

TABLE 5.—*Segregation of F₃ progenies from the cross Niro Vialone × Caloro for color of leaves and leaf sheaths, at Biggs, Calif., 1925*

Color group of F ₂ progeny and number of families	F ₃ progenies having leaves and leaf sheaths—			Deviation from 3:1 ratio, and probable error	Deviation from 9:7 ratio, and probable error *
	Purple	Purple striped	Green		
Purple leaves and leaf sheaths:					
4.....	483		21	-7.50±3.12	
1.....	93		58		-2.00±3.79
1.....	70				
12.....	707	99	464		
Expected in a 27:9:28 ratio *	535.78	178.59	555.63		
Deviation.....	+171.22	-79.59	-91.63		
Purple-striped leaves and leaf sheaths:					
6.....		486	154	-6.00±7.39	
3.....		182	143		+0.81±6.03
Green leaves and leaf sheaths, and green leaves and leaf sheaths with red apexes:					
20.....			2,153		

* Probable errors for number of individuals where only two classes were concerned (see also Tables 7, 8, 12, and 13 and in text) were obtained from tables of probable errors of Mendelian ratios, calculated from the formula $\pm 0.6745 \sqrt{pqn}$, prepared in the Department of Plant Breeding, Cornell University, Ithaca, N. Y.

^b $\chi^2=105.2978$. P =very poor fit.

In none of the segregating F₃ purple families was there produced a ratio of 3 plants with purple leaves and leaf sheaths to 1 purple striped. This probably is due to the fact that an insufficient number of F₃ purple families was grown to include plants of all genetic constitutions. One can hardly expect to get true-breeding families and families that segregate in all four expected ratios from so small a number as 18 families.

Of the nine F₃ families from the purple-striped group, six segregated in the ratio of 3 plants with purple-striped leaves and leaf sheaths to 1 with green, and the remaining three segregated in a ratio of 9 plants with purple-striped leaves and leaf sheaths to 7 with green. Segregation in these ratios was expected, and also true-breeding plants with purple-striped leaves and leaf sheaths. The true-breeding form, however, was not obtained in F₃, but true-breeding plants with purple-striped leaves and leaf sheaths were selected from two of the families in 1925 and were grown in F₄ in 1926. The F₃ results from this group, therefore, are in agreement with expectations.

The data presented regarding the inheritance of color in the leaves and leaf sheaths of the F₁, F₂, and F₃ generations from the cross Niro Vialone × Caloro tend to support the 3-factor hypothesis, but they do not entirely confirm it. While all except one of the expected ratios were obtained in F₃ from the purple and purple-striped F₂ groups, the deviations from the calculated 27:9:28 ratio were large and no doubt significant. Additional support for the 3-factor hypothesis is found, however, in the results from the study of the color inheritance in other organs discussed later in this paper.

COLOR OF AWNS

The color of awns in rice varieties may be either green (straw colored at maturity), yellow, brown, red, purple, or black. Hector (2) and Ikeno (5) found in crosses of rice varieties with colored awns and with green awns that color was dominant in the F₁ hybrids, and

that in F_2 progenies segregation was in the ratio of 3 plants with colored awns to 1 with green. Koch (9, 10) reported that black-awned and pink-awned forms appeared in the progeny from a cross of a white-awned and an awnless variety. In this case the colored awns no doubt were due to the action of complementary factors, one of which may have entered the cross from each variety. Nagai (12) found that in a cross of a brown-awned variety and one with pale yellow awns the awn color in F_1 hybrids was red, and segregation in F_2 was in the ratio of 9 red to 3 brown to 4 pale yellow. Hoshino (4) observed that black awns were dominant to green in F_1 , but he did not report on the segregation for the color of awns in F_2 .

IN NIRO VIALONE \times CALORO

In the cross Niro Vialone \times Caloro, in which Niro Vialone normally is awnless with purple lemma and palea apices, and Caloro has green awns, the F_1 hybrid had a few purplish awns. Although Niro Vialone is normally awnless, it does under certain conditions produce a few short awns. Of the 207 F_2 plants grown, 181 had awns sufficiently developed to study their color. Of the F_3 families grown, one failed to produce awns that were long enough to be included in the study of color.

F_2 PROGENY

In the F_2 progeny the segregation for awn color produced plants in the ratio of 9 purple to 3 red to 4 green, as is shown in Table 6. It is seen in Table 6 that there were 100 plants with purple awns, 25 with red, and 56 with green. The deviations from the expected numbers are small for each group.

TABLE 6.—Segregation of 181 F_2 hybrids from the cross Niro Vialone \times Caloro for color of awns, at Biggs, Calif., 1924

Color of awns	Number of plants—		Deviation
	Observed	Expected in 9:3:4 ratio ^a	
Purple.....	100	101.81	-1.81
Red.....	25	33.94	-8.94
Green.....	56	45.25	+10.75

^a $\chi^2=49.409$. $P=0.0852$.

It was observed that the purple awns often were red and sometimes parti-colored (red and green) upon emergence from the leaf sheaths, but rapidly changed to purple, and that the red awns may lose almost all of the red color by the time the panicles are fully matured. Errors in classification of the red-awned and green-awned groups, therefore, are possible late in the season.

F_3 PROGENY

The purple-awned group consists of the phenotypic classes 1 and 4, the red-awned group of phenotypic classes 2 and 6, and the green-awned group of phenotypic classes 3, 5, 7, and 8. The data on F_3 families in all these groups are shown in Table 7.

From the purple-awned group (class 1) some families in F_3 should breed true for purple awns, and others should segregate in the ratios of 3 purple-awned plants to 1 red-awned plant, 3 purple-awned to 1 green-awned, and 9 purple-awned to 3 red-awned to 4 green-awned. True-breeding families and families segregating approximately in the stated ratios were obtained, as shown in Table 7.

TABLE 7.—Segregation of F_3 progenies from the cross *Niro Vialone* \times *Caloro* for color of awns, at Biggs, Calif., 1925

Color group of F_2 progeny and number of families	F_3 progenies having awns—			Deviation from 3:1 ratio, and probable error
	Purple	Red	Green	
Purple leaves, leaf sheaths, and awns:				
2.....	73			
3.....	210		51	-14.25 \pm 4.71
6.....	63	21		0.00 \pm 2.68
.....	166	64	109	
Expected in a 9:3:4 ratio ^a	190.69	63.66	84.75	
Deviation.....	-24.69	+0.44	+24.25	
Purple-striped leaves and leaf sheaths, and purple awns:				
5.....	266	85		-2.75 \pm 5.47
4.....	182	42	70	
Expected in a 9:3:4 ratio ^b	165.38	55.12	73.50	
Deviation.....	+16.62	-13.12	-3.50	
Green leaves, leaf sheaths, and awns:				
14.....			712	
Green leaves and leaf sheaths, with red awns and lemma and palea apices:				
3.....		142		
2.....		62	29	+6.25 \pm 2.79

^a $\chi^2=10.1386$. $P=0.0064$.

^b $\chi^2=4.9598$. $P=0.0842$.

The expected and observed numbers for the 3:1 ratio are in good agreement. The deviations for the purple-awned and green-awned groups in the 9:3:4 ratio, however, are large, but they probably are not significant and may be due to seedling mortality or possibly to errors in classification.

The purple-striped group (class 4) should segregate for awn color in F_3 in the same manner as the purple group (class 1). The ratios obtained are shown in Table 7. The expected true-breeding purple-awned progeny and the ratio of 3 purple-awned plants to 1 green-awned plant were not obtained in F_3 . This very likely is due to the fact that not enough F_3 families were grown to include plants of all genetic compositions. In F_4 true-breeding purple-striped progenies were observed, which indicates that if more F_3 families had been grown the true-breeding type would have been obtained. The expected and observed numbers for the 3:1 and 9:3:4 ratios obtained from the purple-striped group (class 4) are in good agreement.

The F_3 families from the red-awned group (classes 2 and 6) should produce true-breeding red-awned progeny, and also families that segregate in the ratio of 3 red-awned plants to 1 green-awned plant. Of the six F_3 families grown, 3 bred true for red awns, two segregated in the ratio of 3 red-awned plants to 1 green-awned plant, and one family bred true for awnless plants with red lemma and palea apices. (Table 7.) The results in F_3 for the red-awned group therefore were in accordance with the expectation.

The F_3 families from the green-awned F_2 group (classes 3, 5, 7, and 8) should all breed true for green awns, and all 14 families grown did so.

The purple-awned, red-awned, and green-awned groups obtained in F_2 , with two exceptions, therefore, produced the ratios and the true-breeding types expected in F_3 progenies. The exceptions noted probably are due to the fact that in all groups only a limited number of F_3 progenies was grown, and no doubt all genetic types were not included. This would account for the fact that two expected segregations were not obtained in F_3 . Otherwise the results in F_3 are in fairly good agreement with the expected behavior and tend to confirm the 3-factor hypothesis, and the 2-factor difference for color of awns.

IN BUTTE \times COLUSA

The Butte variety has red awns. Colusa is entirely awnless. In the cross Butte \times Colusa the F_1 plants had red apiculi and red awns.

F_2 PROGENY

The F_2 plants grown in 1925 segregated for awnedness and color of awns. In another study of this cross by the writer (7), 52 awnless and 785 awned and partly awned plants were reported. In this group with awns more or less developed there were 600 plants with red awns to 185 with green awns. The deviation from the expected numbers in a 3:1 ratio was 11.25 plants with a probable error of ± 8.18 . The segregation was in very close agreement, therefore, with the monohybrid 3:1 ratio.

F_3 PROGENY

There were 16 F_2 plants which bred true for green awns in F_3 . Seven of these F_3 families, however, segregated for awnedness, producing some awnless plants. Seven F_2 plants bred true for red awns, and four of these F_3 families segregated for awnedness, producing some awnless plants. Ten F_2 plants with red awns segregated for color of awns in F_3 . There were 301 plants with red awns and 108 with green. The deviation from the expected numbers in a 3:1 ratio was not large for any of the F_3 families. The total number for all families agrees very well with the expected 3:1 ratio. The deviation from the expected ratio is 5.75 plants, and this deviation divided by the probable error, ± 5.91 , is less than unity. The results, therefore, indicate that one genetic factor is involved in the production of awn color in this case. In relation to awn color Butte apparently has the factorial constitution *Cp* and Colusa *cp*.

IN COLUSA \times ITALIAN RED

The Colusa variety is awnless under all conditions and the apiculi are green. Italian Red rice is partly awned, and the awns range in color from purple to almost black. The partly awned F_1 plants from the cross Colusa \times Italian Red had purple awns and lemma and palea apices. Segregation in the F_2 population produced three groups of plants with respect to awn color. Their frequencies are indicated in Table 8.

The awns of F_2 plants often are parti-colored (red and green) when they first emerge from the sheaths, but within a relatively short time such awns either become red or deepen in color from red to purple.

The data on awn color from the cross Colusa \times Italian Red presented in Table 8 agree very well with the numbers expected in a 9:3:4 ratio. The green group shows the largest deviation from the expected ratio. In this cross, color in the awns, therefore, is apparently due to the independent action of two genetic factors.

TABLE 8.—Segregation of F_2 hybrids from the crosses Colusa \times Italian Red and C. I. 5346 \times Italian Red, and from both crosses for color of awns, at Biggs, Calif., 1926

Cross and color group	Number of plants—		Deviation	χ^2	P
	Observed	Expected in a 9:3:4 ratio			
Colusa \times Italian Red:					
Purple.....	198	203.0625	-5.0625	2.3167	0.3220
Red.....	61	67.6875	-6.6875		
Green.....	102	90.2500	+11.7500		
C. I. 5346 \times Italian Red:					
Purple.....	258	287.4375	-29.4375	6.8418	.0333
Red.....	109	95.8125	+13.1875		
Green.....	144	127.7500	+16.2500		
Total from both crosses:					
Purple.....	456	490.5	-34.5	6.2813	.0443
Red.....	170	163.5	+6.5		
Green.....	246	218.0	+28.0		

IN C. I. 5346 \times ITALIAN RED

In the cross C. I. 5346 \times Italian Red the F_1 plants had purple awns, and segregation of F_2 progeny produced three groups of plants with respect to awn color, as shown in Table 8.

Table 8 shows that there is a rather large minus deviation from the expected numbers in the purple group and fairly large plus deviations in the red and green groups. The explanation given below for the deficiency and excess of color in the lemma and palea apexes in the same cross also applies in this case. In each of these cases the deviation from the expected numbers in the 9:3:4 ratio is not exceedingly large, and the data indicate that in all probability the varieties used in this study of awn color differ by two independently inherited genetic factors.

Table 8 shows the combined data from the segregation in F_2 progeny for color of awns in the two crosses last discussed. It is observed that the deviations from the expected numbers in a 9:3:4 ratio are rather large for the purple and green groups. It is the opinion of the writer, however, that these deviations probably are due to errors in classification of F_2 plants and that the results can be explained on the basis of a 2-factor difference for awn color.

The data presented on the inheritance of awn color indicate that Italian Red has the factorial constitution *CP*, and Colusa and C. I. 5346, *cp*.

COLOR OF APICULI AND OF LEMMA AND PALEA APEXES

As previously noted, the term "apiculi" refers to the excurrent ends of fibrovascular bundles of the lemma and palea, whereas the term "lemma and palea apexes" includes not only the apiculi but also the immediately adjacent portion of these organs.

The color of the lemma and palea apices is a character often used in classifying rice varieties. The apices of the lemma and palea of Japanese rices most commonly are green or straw colored at maturity, but they may be brown, red, purple, or purplish black. Most rice varieties grown in the United States have green lemma and palea apices, which become straw colored at maturity.

Hector (2, 3) found in crosses between a variety with colored apiculi and one with green that in the F_1 hybrids they were colored, and segregation in F_2 resulted in ratios of 3:1, 9:7, 15:1, and 27:37 plants with colored and green apiculi, respectively. Ikeno (5) and Parnell and others (13) found color in the apiculi dominant in F_1 , and segregation in F_2 produced 3 colored plants to 1 green plant. These results indicate that colored apiculi may be due to a single-factor difference or to two or three dominant complementary factors or to dominant duplicate factors.

IN NIRO VIALONE \times CALORO

The Italian variety Niro Vialone has purple or purplish lemma and palea apices, whereas those of Caloro are green. In the F_1 hybrid from the cross Niro Vialone \times Caloro the lemma and palea apices were purple.

F_2 PROGENY

Segregation in F_2 produced plants in the ratios of 9 with purple apices to 3 with red to 4 with green. The number of plants in each group is shown in Table 9. The deviations from the expected numbers for each group are small.

As the color of the lemma and palea apices and awns in the cross Niro Vialone \times Caloro apparently is due to the same factors, and as the purple, red, and green groups for apices are composed of the same phenotypic classes as those for awn color, it seems unnecessary to report here the detailed results obtained in F_3 on the inheritance of color of the lemma and palea apices. The two expected ratios which were not obtained for awn color were not obtained for apex color. The possible reasons for this are the same as those discussed in the case of awn color. The differences in total number of plants reported in the study of awns and apices in F_3 are due to the fact that some families were awnless but segregated for color of apices.

TABLE 9.—Segregation of 207 F_2 hybrids from the cross Niro Vialone \times Caloro for color of lemma and palea apices, at Biggs, Calif., 1924

Color of apices	Number of plants*		Deviation
	Observed	Expected in 9:3:4 ratio	
Purple.....	115	116.44	-1.44
Red.....	30	38.81	-8.81
Green.....	62	51.75	+10.25

* $\chi^2=4.0479$. $P=0.1328$.

F_3 PROGENY

In the F_3 progeny 4 F_2 plants having purple apices, 14 having green apices, and 3 having red apices bred true. All expected ratios and true-breeding forms in F_3 were obtained, as is shown in

Table 10. The expected and observed numbers agree very well for all groups, except for the 9:3:4 ratios. Since the expected and observed numbers agree fairly well for all other groups, these deviations may not be significant. The results indicate that two genetic factors are involved in the development of color in the apexes.

TABLE 10.—Segregation of F_3 progenies from the cross *Niro Vialone* × *Caloro* for color of lemma and palea apexes, at Biggs, Calif., 1925

Color group of F_3 progeny and number of families	F_3 progenies having apexes—			Deviation from 3:1 ratio, and probable error
	Purple	Red	Green	
Purple leaves, leaf sheaths, and lemma and palea apexes:				
4.....	483	-----	-----	-----
3.....	226	-----	64	-8.50±4.97
4.....	342	126	-----	+9.00±6.32
7.....	408	121	222	-----
Expected in a 9:3:4 ratio ^a	422.44	140.81	187.75	-----
Deviation.....	-14.44	-19.81	+34.25	-----
Purple-striped leaves and leaf sheaths, and purple lemma and palea apexes:				
5.....	379	128	-----	+1.25±6.58
4.....	294	69	95	-----
Expected in a 9:3:4 ratio ^b	257.63	85.87	114.50	-----
Deviation.....	+36.37	-16.87	-19.50	-----
Green leaves, leaf sheaths, and lemma and palea apexes:				
14.....	-----	-----	1,471	-----
Green leaves and leaf sheaths with red awns and lemma and palea apexes:				
3.....	-----	371	1	-----
3.....	-----	224	86	+8.50±5.14

^a $\chi^2=9.5286$ $P=0.0088$.

^b $\chi^2=11.7697$ $P=0.0028$.

^c The seed producing this plant floated in during the first irrigation.

IN BUTTE × COLUSA

The Butte variety has reddish apiculi, whereas Colusa has green. In the cross Butte × Colusa the F_1 plants had red apiculi, but the adjacent tissues of the apexes were green.

F_2 PROGENY

In the F_2 progenies grown in 1925 there were 634 plants with red to 203 with green apiculi. These numbers are in close agreement with those expected in a 3:1 ratio. The deviation is only 6.25 plants, with a probable error of ± 8.45 . The data indicate that there is a single genetic-factor difference for color of the apiculi in this cross.

F_3 PROGENY

In F_3 , 10 of the families bred true for red and 19 for green apiculi, just as they did for red and green awns, as was expected. However, 11 families segregated for red and green apiculi in F_3 . There were 404 plants with red apiculi and 152 with green.

The segregation of nearly all families agreed very well with that expected in a 3:1 ratio. The deviation from the expected numbers in a 3:1 ratio was 13 plants, with a probable error of ± 6.89 . The observed and expected numbers are in close agreement, therefore, which indicates that one factor is involved in the production of red apiculi in the rices here studied. Apparently the same genetic factor is responsible for red apiculi and red awns in this cross.

IN COLUSA \times ITALIAN RED

Colusa normally has no color in any of its organs. In Italian Red many of the organs, including the lemma and palea apices, are purplish. In the cross Colusa \times Italian Red the lemma and palea apices were purple in the F_1 . The F_2 plants were separated into three groups, purple, red, and green. The results are shown in Table 11. The observed numbers of plants agreed very well with the expected numbers for each color group. The deviation from the numbers expected in the 9:3:4 ratio is very small in each group. These data indicate that two independently inherited factors account for the production of color in the lemma and palea apices in this case.

IN C. I. 5346 \times ITALIAN RED

Data on the segregation of F_2 plants of the cross C. I. 5346 \times Italian Red for color of the lemma and palea apices are presented in Table 11.

TABLE 11.—Segregation of F_2 hybrids from the crosses Colusa \times Italian Red, and C. I. 5346 \times Italian Red, and from both crosses and from the cross Niro Vialone \times Wataribune, for color of lemma and palea apices, at Biggs, Calif., 1926

Cross and color group	Number of plants		Deviation	χ^2	P
	Observed	Expected in 9:3:4 ratio			
Colusa \times Italian Red:					
Purple.....	291	283	+3	0.2683	0.8944
Red.....	98	96	+2		
Green.....	123	128	-5		
C. I. 5346 \times Italian Red:					
Purple.....	259	289.125	-30.125	7.2278	.0275
Red.....	111	96.375	+14.625		
Green.....	144	128.500	+15.500		
Total from both crosses:					
Purple.....	550	577.125	-27.125	3.1414	.2107
Red.....	209	192.375	+16.625		
Green.....	267	256.500	+10.500		
Niro Vialone \times Wataribune:					
Purple.....	286	264.375	+21.625	11.1046	.0039
Red.....	60	88.125	-28.125		
Green.....	124	117.500	+6.500		

It will be observed that the deviations from the expected numbers in a 9:3:4 ratio are larger than for the progeny of Colusa \times Italian Red. The deviation for the purple group is negative and relatively large. These F_2 plants were classified when they were nearly all mature, and it is quite probable that on some of the plants the purple color of the lemma and palea apices had faded to such an extent that they were not correctly classified, as both the red and the green groups have rather large plus deviations. The writer often has observed that the purple or red color in various plant organs may disappear almost entirely when the plants reach maturity, and for this reason it is very difficult to classify correctly an F_2 population late in the season. The results indicate, however, that two independent genetic factors probably are responsible for color in the lemma and palea apices.

In Table 11 are combined the results of F_2 segregation for color of lemma and palea apexes in both crosses. The combined numbers agree very well with those expected in a 9:3:4 ratio. This indicates that color in the lemma and palea apexes of Italian Red rice is due to two independently inherited genetic factors. The data indicate that the factorial constitution with respect to color of lemma and palea apexes is *CP* for the Italian Red variety and *cp* for Colusa and C. I. 5346.

IN NIRO VIALONE \times WATARIBUNE

In Niro Vialone nearly all the organs are purplish, whereas in Wataribune none is colored. In the cross of these two, purple lemma and palea apexes were dominant in F_1 . Segregation in F_2 resulted in a ratio of about 9 plants with purple lemma and palea apexes to 3 plants with red to 4 plants with green, as shown in Table 11. The deviation from this ratio may be significant, for the value of P indicates a rather poor fit.

IN EUREKA \times CALORO

Eureka has no colored organs except the apiculi, which are red. All the organs of Caloro are without color. In the cross between these two varieties the F_1 plants had red apiculi. The segregation of F_2 plants resulted in approximately 3 plants with red to 1 with green apiculi. From certain segregating F_3 families there again were obtained approximately 3 plants with red apiculi to 1 with green, thus verifying the correctness of the monohybrid ratio. However, in two F_3 families, there were two types of red apiculi. In one the red developed early, that is, as soon as the plants began to head; whereas in the other the apiculi were green until the spikelets began to ripen. Then the apiculi assumed a brick-red color. In these two families the ratio of red to brick red was 3:1.

COLOR OF INTERNODES, GLUMES, AND STIGMAS

The internodes in rice usually are green, but in some varieties they are colored. Parnell and others (13) reported that in a cross of a variety having golden internodes with one having green, golden was recessive to green, and in F_2 there were 3 plants with green internodes to 1 with golden. He attributed the dominance of the green color in this case to the action of a factor which inhibited the appearance of the golden color. In another cross Parnell and his associates found that purple internodes were dominant in F_1 and that segregation in F_2 produced 3 plants with purple internodes to 1 with green.

Hector (3) found that colored internodes and stigmas were dominant to green in F_1 , and that segregation in F_2 occurred in ratios of 3:1, 9:7, and 27:37 colored to green plants, respectively. In one case Hector (2) found that stigma color was due to the interaction of four dominant complementary factors giving in F_2 81 plants with colored stigmas to 175 with green. He found also that colored glumes were dominant over green in F_1 , and that in the F_2 progenies ratios of 3 plants with colored glumes to 1 with green, or 9 with colored to 7 with green, were obtained. Ikeno (5) reported a 3:1 ratio in F_2 for plants with colored and green stigmas. These data indicate that color in the internodes and stigmas may be due to the action of one genetic

factor, or to the interaction of two or three dominant complementary factors, while color in the glumes may be due to a single genetic factor or to complementary factors.

IN NIRO VIALONE \times CALORO

In Niro Vialone, it will be remembered, nearly all the organs, including the three under discussion, are purple or purplish. In Caloro, on the other hand, all the organs are green. In the cross between Niro Vialone and Caloro the F_1 hybrids had purple internodes, glumes, and stigmas.

F_2 PROGENY

Segregation in F_2 produced two groups, consisting of 115 plants with purple internodes, glumes, and stigmas and 92 plants with these organs green. The numbers expected in a 9:7 ratio are 116.44 and 90.56, respectively. The deviation is 1.44 plants, with a probable error of ± 4.81 .

F_3 PROGENY

The purple group consists of phenotypic classes 1 and 4, and the green groups of phenotypic classes 2, 3, 5, 6, 7, and 8. In F_3 progenies from the purple group some families should breed true for purple internodes, glumes, and stigmas, while others should segregate in the ratio of 3 plants with these organs colored to 1 with organs green, and 9 with colored organs to 7 with green. Families in the green group, regardless of genetic constitution, should breed true in F_3 , and this result was obtained in 20 families, consisting of 2,153 plants. The results are shown in Table 12.

TABLE 12.—Segregation of F_3 progenies from the cross Niro Vialone \times Caloro for color of internodes, glumes, and stigmas, at Biggs, Calif., 1925

Color group of F_2 progeny and number of families	F_3 progenies having internodes, glumes, and stigmas—		Deviation from 3:1 ratio, and probable error	Deviation from 9:7 ratio, and probable error
	Purple	Green		
Purple leaves and leaf sheaths, internodes, glumes, and stigmas:				
4-----	483			
7-----	568	190	+0.50 \pm 8.04	
7-----	405	349		+19.13 \pm 9.19
Purple-striped leaves and leaf sheaths, and purple internodes, glumes, and stigmas:				
6-----	487	153	-7.00 \pm 7.39	
3-----	185	140		-2.19 \pm 6.03
Green leaves and leaf sheaths, and green leaves and leaf sheaths with red apices, both groups with green internodes, glumes, and stigmas:				
20-----		2,153		

An inspection of Table 12 shows that in the families from class 1 of the purple group four families contained only individuals with purple organs, and that the observed and expected numbers for the 3:1 and 9:7 ratios are in excellent agreement. One expected ratio is lacking, however, for there should be some families which in segregation would produce 3 plants with purple internodes, glumes, and stigmas to 1 with those organs red. No plants with red internodes, glumes,

and stigmas were detected in F_3 , however. It is possible that such plants were present and that the red color was so dilute that it escaped detection. In this event the red plants would be classed as green, but as all plants were carefully classified this hardly seems possible. This particular segregation is lacking also from class 4 of the purple group.

No true-breeding purple progenies were obtained in F_3 from class 4 of the purple group, but true-breeding strains were present in F_4 . Six of the nine segregating F_3 progenies from class 4 produced about 3 plants with purple internodes, glumes, and stigmas to 1 plant with green. The other three families produced plants in the ratio of 9 purple to 7 green, as shown in Table 12. The observed and expected numbers for these ratios in F_3 are in very good agreement.

The data herein presented on the inheritance of color of the internodes, glumes, and stigmas, in the cross Niro Vialone \times Caloro, are in general agreement with the 3-factor hypothesis. The colors of internodes, glumes, and stigmas in this case apparently are due to the same dominant complementary genetic factors or to completely linked factors.

IN COLUSA \times ITALIAN RED

Colusa has no colored organs, but Italian Red rice has internodes with purplish color extending up and down the culms for a short distance from the nodes. In the cross Colusa \times Italian Red the F_1 plants have purple internodes, and in F_2 there were 226 plants with purple internodes to 280 with green internodes. These numbers agree very well with a Mendelian 27:37 ratio. The deviation from the expected numbers is 12.53 ± 7.49 plants.

The F_1 plants from this cross also had purplish color in the glumes and stigmas. The segregation in F_2 produced 292 plants with purple glumes and stigmas to 222 with green. The deviation from the expected numbers in a 9:7 ratio is 2.875 plants with a probable error of ± 7.59 , which indicates a very good fit.

Attention should be called to the fact that in the cross Niro Vialone \times Caloro the color of the internodes, glumes, and stigmas was inherited as a unit, whereas in the cross Colusa \times Italian Red the color in the internodes appears to be due to three complementary factors and that in the glumes and stigmas to two complementary factors.

IN NIRO VIALONE \times WATARIBUNE

The variety Niro Vialone, having nearly all organs purple, and the variety Wataribune, having all organs green, were crossed. In this cross the F_1 plants had purple internodes, and segregation in F_2 produced 286 plants with purple internodes to 184 with green ones. These numbers are in fair agreement with those expected in a 9:7 ratio. The results presented indicate that color in the internodes of the rices studied apparently is due to the presence of two or three dominant complementary factors.

COLOR OF NODES, LIGULES, AURICLES, AND PULVINI

In rice the nodes, ligules, auricles, and pulvini usually are green. In some varieties these organs are colored, however. Hector (3) found in rice crosses that color in the ligules, auricles, and pulvini was

dominant to green in F_1 , and that segregation in F_2 resulted in approximately 9 plants with these organs colored to 7 in which they were green. He also reports in the F_2 segregation of one cross a ratio of 27 plants with colored ligules to 37 with green. These results indicate that color in the ligules, auricles, and pulvini of the rice studied by Hector was due to the interaction of two dominant complementary genetic factors, and that in one case ligule color was due to the interaction of three dominant factors. Parnell and others (13) found that purple color in auricles and pulvini was dominant in F_1 and that segregation in F_2 produced three plants with purple auricles and pulvini to one with green.

A great deal of difficulty is experienced in studying the inheritance of color of nodes in rice. In California the nodes of the rice culms often remain inclosed by the leaf sheaths from the next lower nodes and, therefore, are not exposed to the direct rays of the sun. Exposure to the sun appears to be necessary, however, for the proper development of color in the nodes; therefore errors in classification scarcely can be avoided, even if the sheaths are removed and the naked nodes studied.

IN NIRO VIALONE \times CALORO

Niro Vialone has purple nodes, ligules, auricles, and pulvini, whereas Caloro has no colored organs. In the F_1 hybrid from the cross Niro Vialone \times Caloro all of these organs were purple.

F_2 PROGENY

Segregation in F_2 resulted in 100 plants with these organs purple to 107 plants with them green. The numbers calculated for a 27:37 ratio are 87.53 and 119.67, respectively, and the deviation is 12.67 plants, with a probable error of ± 4.79 . The deviation therefore is somewhat less than three times the probable error, which indicates a satisfactory fit.

F_3 PROGENY

The purple group consists of phenotypic class 1, and the green group of phenotypic classes 2 to 8, inclusive. On a 3-factor basis some of the purple-group progenies should breed true in F_3 and others should segregate in ratios of 3 purple plants to 1 green, 9 purple plants to 7 green, and 27 purple plants to 37 green. All F_3 progenies from the green group should and did breed true, 20 families, containing 2,153 plants, being tested in this group. The data on F_3 progenies are presented in Table 13.

Of the 18 F_3 progenies from the purple group 4, consisting of 483 plants, bred true for purple nodes, ligules, auricles, and pulvini. Three progenies segregated in the ratio of 3 plants with these organs purple to 1 with green. Eight progenies segregated in the ratio of 9 with purple to 7 with green, and 3 progenies segregated in the ratio of 27 with purple to 37 with green. Nine F_2 families having purple-striped leaves and leaf sheaths and green nodes, ligules, auricles, and pulvini, consisting of 965 plants, bred true for green nodes, ligules, auricles, and pulvini.

TABLE 13.—Segregation of F_3 progenies from the cross Niro Vialone \times Caloro for color of nodes, ligules, auricles, and pulvini, at Biggs, Calif., 1925

Color group of F_2 progeny and number of families	F_3 progenies having nodes, ligules, auricles, and pulvini—		Deviation from 3:1 ratio, and probable error	Deviation from 9:7 ratio, and probable error	Deviation from 27:37 ratio, and probable error
	Purple	Green			
Purple leaves, leaf sheaths, nodes, ligules, auricles, and pulvini:					
4.....	483				
3.....	259	103	+12.50 \pm 5.56		
8.....	475	370		+0.31 \pm 9.73	
3.....	137	168			+8.33 \pm 5.82
Purple-striped leaves and leaf sheaths and green nodes, ligules, auricles, and pulvini:					
9.....		965			
Green leaves and leaf sheaths, and green leaves and leaf sheaths with red apexes, both groups with green nodes, ligules, auricles, and pulvini:					
20.....		2,153			

An inspection of Table 13 shows that the observed and expected numbers for each of the three ratios are in excellent agreement, which indicates that for the color of nodes, ligules, auricles, and pulvini, the 3-factor hypothesis fits the results. Color in these four organs in this case apparently is due to the interaction of three dominant complementary factors. The color in all four organs is inherited as a unit, and the color of each apparently is due to the same genetic factor or factors in the variety here studied.

The data presented in the preceding pages on the inheritance of color in the cross Niro Vialone \times Caloro were found, as a whole, to warrant the 3-factor hypothesis. A few ratios that were expected in F_3 were not obtained, but this probably was due to the limited number of F_3 progenies grown and to the large number of characters studied. One true-breeding type not obtained in F_3 was found to be present in the F_4 selections. The deviation from the expected ratio in F_3 was in one case very large, and no doubt significant, but in nearly all other cases the observed and expected numbers were in fairly good agreement. In a study of so many characters a much larger number of F_3 families and plants should have been used, but this was not possible because of the shortage of land and labor. It is felt, however, that the data presented, while not extensive, have been in harmony with the 3-factor hypothesis.

Color in the rice plant was found by Hector (3) and Nagai (12) to be inherited by groups of organs. In the cross Niro Vialone \times Caloro, likewise, the color characters of the Niro Vialone parent, as has been noted, were inherited in groups as if the colors were due to the action or interaction of the same genetic factors or to completely linked factors. In this cross color was inherited by four groups of organs, namely: (1) Leaves and leaf sheaths; (2) awns and lemma and palea apexes; (3) internodes, glumes, and stigmas; and (4) nodes, ligules, auricles, and pulvini. The colors of all organs within each group were inherited in the F_2 and F_3 generations as if they were due to the same genetic factors or to completely linked factors, but no evidence was found in support of completely linked factors.

IN COLUSA \times ITALIAN RED

Colusa has no colored organs, whereas Italian Red has purple color in all the organs under consideration. In this cross the F_1 plants had culms with purple nodes. Of the F_2 plants, 254 had purple nodes and 253 had green nodes. These numbers, being almost equal, represent practically a perfect 1:1 ratio, but such a ratio is not to be expected from an F_1 population in which purple nodes were dominant. It is very possible, therefore, that some of the F_2 plants classed as having green nodes would have developed color in the nodes with longer exposure to the sun. This probably would have resulted in a 9:7 ratio with purple nodes dominant.

In the cross Colusa \times Italian Red the colors of the ligules, auricles, and pulvini were inherited as if they were due to the same factors or to completely linked factors. There were 269 F_2 plants with purple ligules, auricles, and pulvini to 244 with these organs green. These numbers are in fairly good agreement with a 9:7 ratio. The deviation from the expected numbers in a 9:7 ratio is 19.56 plants with a probable error of ± 7.58 . The deviation probably is not significant, for it is less than three times the probable error. The purple color in these organs apparently is due to the complementary action of two dominant genetic factors, both of which are necessary for the production of the color.

IN NIRO VIALONE \times WATARIBUNE

In the plants from a cross between Niro Vialone and Wataribune grown at Biggs, the nodes were colored in F_1 . Niro Vialone has purplish and Wataribune green nodes. Of the F_2 plants, 258 had purple nodes and 212 had green. This is in close agreement with the numbers expected in a 9:7 ratio. The deviation from the expected is 6.375 plants, with a probable error of ± 7.25 .

COLOR OF SEED COATS

The color of the seed coats, as seen in hulled kernels of rice, may be white, brown, red, purple, or purplish black. White, or brown of various shades, are the most common colors. Red rice is present in most rice-growing countries, but purple rice is rather uncommon.

Red rice derives its name from the red color of the outer seed coats, visible in hulled kernels. The intensity of the color depends, for a given variety, upon the stage of maturity of the rice. Usually only well-matured kernels are fully red. There probably are varieties of rice which represent each shade of kernel color between white and dark red. In most red varieties the color is confined to the surface coats of the kernels. The color in some red rices appears, however, to extend into the pericarp.

The inheritance of red color in rice kernels has been studied by several workers. Hector (1) and Parnell and others (13, 14) in India, McKerral (11) and Thompson (17) in Burma, Van der Stok, according to McKerral (11), in Java, Ikeno (5) and Kato and Isikawa (8) in Japan, and Jacobson (6) in the Philippines, all reported that red color is dominant in F_1 and that Mendelian ratios of 3 red-kernelled plants to 1 white-kernelled plant occur in F_2 or later generations.

Parnell and others (13, 14), in a study of the inheritance of color in rice, found F_2 ratios of 3 colored-kernelled plants to 1 white-ker-

neled and of 9 red-kerneled plants to 3 gray-brown to 4 white-kerneled ones. Kato and Isikawa (8) also reported, in the F_2 of one cross, a ratio of 3 red-kerneled plants to 1 white-kerneled. In another cross, using the same red female parent, but a different white male parent, they found that the segregation in F_2 was in the ratio of 9 plants having red kernels to 3 having yellowish-brown kernels to 4 having white kernels. Nagai (12), in a cross of a variety having pale-buff (white) kernels with one having reddish-brown kernels, reported segregation in F_2 in the ratio of 9 reddish-brown to 3 yellow-brown to 4 pale-buff (white) plants. Kato and Isikawa (8) also found that the reddish and yellowish-brown pigments in the rices studied by them belong to the protoeyan and not to the anthocyan group of pigments.

IN COLUSA \times ITALIAN RED AND C. I. 5346 \times ITALIAN RED

Colusa and C. I. 5346 have no colored organs, whereas Italian Red has purplish color in most organs and has red seed coats. Three F_1 plants of the cross Colusa \times Italian Red and seven F_1 plants of the cross C. I. 5346 \times Italian Red were grown to maturity. The F_1 plants of both crosses appeared to be more vigorous than the parent varieties. The Colusa variety matured from September 27 to 29, C. I. 5346 September 23 to 24, and Italian Red September 20 to 21. The F_1 plants of Colusa \times Italian Red matured September 23, and those of C. I. 5346 \times Italian Red September 21. The F_1 plants of both crosses, therefore, matured more nearly with the early parent.

The F_1 plants of the crosses Colusa \times Italian Red and C. I. 5346 \times Italian Red had color in all the organs that normally are colored in the male parent, though in most of the parts the color was less highly developed. The nodes, lemma and palea apices, and awns were nearly as highly colored, and the hulled F_1 kernels were nearly as red, as in the male parent.

F_2 PROGENY

The counts on kernel color in the crosses studied were made late in October, when nearly all F_2 plants were well matured. A few very late F_2 plants did not mature well enough to make possible a classification of the color of the kernels, for the red color of the hulled kernel is not fully developed in California except in well-matured rice. The color of the kernels of the mature F_2 plants varied from white through pale red to dark red. It is probable that the dark-red kernels were on homozygous F_2 plants, and those with the lighter shades of red were on heterozygous F_2 plants. In these crosses all red kernels, regardless of the intensity of the red color, were classed as red.

In the cross Colusa \times Italian Red there were 381 F_2 plants with red kernels to 120 F_2 plants with white kernels. These numbers are in close agreement with the Mendelian 3:1 ratio. The deviation from the ratio of 3 red to 1 white is only 5.25 plants, with a probable error of ± 6.54 .

In the cross C. I. 5346 \times Italian Red there were 400 F_2 red-kerneled plants to 125 with white kernels. These numbers also are in close agreement with a 3:1 ratio, with red-kernel color dominant. The deviation from a 3:1 ratio in this case is 6.25 plants and the probable error ± 6.69 . The segregation in F_2 for both crosses gave a total of 781 plants with red seeds to 245 plants with white seeds,

which is in very close agreement with the Mendelian monohybrid 3:1 ratio. The deviation from the expected number in a 3:1 ratio is only 11.5 plants, with a probable error of ± 9.36 . It appears therefore that the red-kerneled and white-kerneled rices used as parents in these crosses differ by only a single genetic factor.

SUMMARY

In the crosses Niro Vialone \times Caloro, Butte \times Colusa, Colusa \times Italian Red, Eureka \times Caloro, and an unnamed variety C. I. 5346 \times Italian Red, all organs that were colored in the Niro Vialone, Butte, Eureka, and Italian Red varieties were colored in the F_1 hybrids.

In F_2 , segregation for purple color of the leaves and leaf sheaths, in the cross Niro Vialone \times Caloro, produced numbers fairly close to a 27:9:28 ratio. In F_3 , the green strains bred true and the purple strains either bred true or segregated according to their genetic constitution in 3:1, 9:7, or 27:9:28 ratios. In the 27:9:28 ratio the fit was poor.

The F_2 segregation for color of the awns and lemma and palea apices in the crosses Niro Vialone \times Caloro, Colusa \times Italian Red, and C. I. 5346 \times Italian Red produced numbers agreeing with a 9:3:4 ratio. In F_2 , Niro Vialone \times Wataribune produced plants with purple, red, and green lemma and palea apices in the ratio of approximately 9:3:4. The Butte \times Colusa cross segregated in F_2 for color of both awns and apiculi in a ratio of 3 red to 1 green. The correctness of the last ratio was verified by growing F_3 progenies. In F_3 segregation, in the cross Niro Vialone \times Caloro, the green strains bred true and the colored strains either bred true or segregated in 3:1 or 9:3:4 ratios.

The F_2 segregation in the cross Niro Vialone \times Caloro for color of the internodes, glumes, and stigmas was in a 9:7 ratio of purple to green. In F_3 , the green strains bred true and the purple strains either bred true or segregated in 3:1 or 9:7 ratios. The F_2 segregation in the cross Colusa \times Italian Red produced plants with purple and green internodes in a 27:37 ratio and plants with purple and green glumes and stigmas in a 9:7 ratio. The F_2 segregation in the cross Niro Vialone \times Wataribune produced plants with purple and green internodes in a 9:7 ratio.

The F_2 segregation in the cross Niro Vialone \times Caloro for color of the nodes, ligules, auricles, and pulvini produced plants with these organs purple and green in the ratio of 27:37. In F_3 , the green strains bred true and the purple strains either bred true or segregated in 3:1, 9:7, or 27:37 ratios. The F_2 segregation in the cross Colusa \times Italian Red for color of the ligules, auricles, and pulvini produced plants with these organs purple and green in a 9:7 ratio, while the plants with purple nodes and those with green appeared in about equal numbers. The F_2 segregation in the cross Niro Vialone \times Wataribune for color of nodes produced plants with purple and green nodes in the ratio of 9:7.

In the cross Niro Vialone \times Caloro the colors of the four groups of colored organs—leaves and leaf sheaths; awns and lemma and palea apices; internodes, glumes, and stigmas; and nodes, ligules, auricles, and pulvini—were inherited separately. Color in each group was inherited as a unit, however, and as if it were due to the same genetic factors or to completely linked factors. The results indicate that it is due to the same and not to completely linked factors.

The data presented indicate that with respect to the colored vegetative organs the Niro Vialone parent has the dominant complementary factors *ACP* and the Caloro parent the recessive factors *acp*. The interaction of these three factors gives the colors observed in the four groups of organs mentioned above. No color develops in the absence of factor *C*.

In the cross Colusa \times Italian Red, the color of the awns, of the lemma and palea apexes, of the glumes and stigmas, and of the ligules, auricles and pulvini, was inherited in each group of organs as if it were due to the same genetic factors or to completely linked factors. The color of awns and apiculi and of lemma and palea apexes in the crosses Butte \times Colusa and C. I. 5346 \times Italian Red also was inherited as a unit.

Purple color in the organs studied was found to be bigenic; that is, two (or more) factors acting together were necessary for the production of purple color in all organs.

The F_2 segregation, in the crosses Colusa \times Italian Red and C. I. 5346 \times Italian Red, for color of the seed coats, as seen in hulled kernels, produced approximately 3 red-kerneled plants to 1 white-kerneled plant.

LITERATURE CITED

- (1) HECTOR, G. P.
1913. NOTES ON POLLINATION AND CROSS-FERTILISATION IN THE COMMON RICE PLANT, *ORYZA SATIVA*, LINN. India Dept. Agr. Mem., Bot. Ser. 6: 1-10.
- (2) ———
1916. OBSERVATIONS ON THE INHERITANCE OF ANTHOCYAN PIGMENT IN PADDY VARIETIES. India Dept. Agr. Mem., Bot. Ser. 8: [89]-101, illus.
- (3) ———
1922. CORRELATION OF COLOUR-CHARACTERS IN RICE. India Dept. Agr. Mem., Bot. Ser. 11: 153-183, illus.
- (4) HOSHINO, Y.
1915. ON THE INHERITANCE OF FLOWERING TIME IN PEAS AND RICE. Jour. Col. Agr., Tohoku Imp. Univ. 6: [229]-288, illus.
- (5) IKENO, S.
1919. ZIKKEN-IDENGAKU. [A TEXT BOOK ON GENETICS]. Ed. 3, rewritten and augmented. 230 p. illus., 1918. (Abstract) Bot. Abs. 2: 114.
- (6) JACOBSON, H. O.
1914. XENIA (?) IN RICE. Philippine Agr. Rev. 7: 361-362.
- (7) JONES, J. W.
1927. INHERITANCE OF AWNEDNESS IN RICE. Jour. Amer. Soc. Agr. 19 (9): 830-839, illus.
- (8) KATO, S., and ISIKAWA, Z.
1923. [ON THE HEREDITY OF THE PIGMENTS OF RED RICE.] [Japanese original not seen.] (Abstract) Japan. Jour. Bot. 1: (5)-(6).
- (9) KOCH, L.
1916. HET PLANTEN VAN CASSAVE VOLGENS DE METHODE VAN HEEMSTEDÉ OBELT VERGELEKEN MET DE GEWONE BIJ DE BEVOLKING IN ZWANG ZIJNDE METHODEN. Teysmannia 27: [240]-245.
- (10) ———
1916. DE BETEKENIS VAN DE BASTAARDSELECTE BIJ PADI, EN HOE DEZE WORDT UITGEVOERD. Teysmannia 27: [502]-519, illus.
- (11) McKERRAL, A.
1913. SOME PROBLEMS OF RICE IMPROVEMENT IN BURMA. Agr. Jour. India 8: [317]-330.
- (12) NAGAI, I.
1921-23. A GENETICO-PHYSIOLOGICAL STUDY ON THE FORMATION OF ANTHOCYAN AND BROWN PIGMENTS IN PLANTS. Jour. Col. Agr., Imp. Univ. Tokyo 8: [1]-92, illus.

- (13) PARNELL, F. R., RANGASWAMI AYYANGAR, G. N., and RAMIAH, K.
1917. THE INHERITANCE OF CHARACTERS IN RICE. I. India Dept. Agr.
Mem., Bot. Ser., 9: 75-105, illus.
- (14) ——— RANGASWAMI AYYANGAR, G. N., RAMIAH, K., and SRINIVASA
AYYANGAR, C. R.
1922. THE INHERITANCE OF CHARACTERS IN RICE. II. India Dept. Agr.
Mem., Bot. Ser. 11: 185-208, illus.
- (15) ROY, S. C.
1921. A PRELIMINARY CLASSIFICATION OF THE WILD RICES OF THE CENTRAL
PROVINCES AND BERAR. Agr. Jour. India 16: 365-380.
- (16) TAKEZAKI, Y.
1923. [UEBER DIE VERERBUNG DER BLATTFARBE BEI DEN PURPURNEN
REISPFLANZEN.] [Japanese original not seen.] (Abstract)
Japan. Jour. Bot. 1: (14).
- (17) THOMPSTONE, E.
1915. SOME OBSERVATIONS ON UPPER BURMA PADDY (GROWN UNDER
IRRIGATION). Agr. Jour. India 10: 26-53, illus.

THE COMPARATIVE NUTRITIVE VALUE OF YELLOW CORN AND THE GRAIN SORGHUMS HEGARI AND YELLOW MILO¹

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INTRODUCTION

The sorghum grains are of great economic importance in the arid Southwest. They are immigrant crops, native to South Africa, which have proved themselves to be well adapted for growth in the south-western plains, for they are drought resistant and high yielding with a minimum amount of water (19).² For this reason they are widely used in animal feeding rations in place of yellow corn, which can not be successfully grown in the drier regions (11).

Dissatisfaction has frequently accompanied the use of the sorghum grains as substitutes for yellow corn in farm-animal rations, the stock feeders thereby losing confidence in the nutritive value of these grains. Reports of "nutritional roup" in poultry following the use of these grains in Arizona are numerous. As a part of a hog-feeding investigation conducted by the Arizona Agricultural Experiment Station,³ ground hegari was compared with yellow corn in the feeding of two lots of pigs for a period of 21 weeks. The pigs fed the corn were reported thrifty and vigorous, whereas those receiving the hegari showed evidences of some nutritional deficiency, failed to conceive, and became deaf and blind. These results, however, have not been duplicated in later experimental work.

Only a very limited number of tests of the nutritive value of the grain sorghums have been reported, the most recent and comprehensive being the study of the chemical and nutritive properties of a wide range of sorghum grains conducted by Heller and Green at the Oklahoma experiment station in 1925 (3). The present paper reports further upon the comparative nutritive value of yellow corn and the grain sorghums hegari and dwarf yellow milo.

EXPERIMENTAL DATA

CHEMICAL ANALYSES

Selected samples of hegari, dwarf yellow milo, and yellow corn were supplied by the agronomy department of the University of Arizona. The hegari and milo were grown on the university experimental farm and the yellow corn in Riley County, Kans. The results of routine chemical analyses of samples of the three grains used appear in Table 1.

¹ Received for publication July 24, 1929; issued June, 1930.

² Reference is made by number (italic) to "Literature cited," p. 1144.

³ WILLIAMS, R. H., BURNS, R. H., and SMITH, C. A. Unpublished data. Department of Animal Industry, University of Arizona. 1923.

TABLE 1.—Percentage analyses of air-dry samples of hegari, dwarf yellow milo, and yellow corn

Grain	Moisture	Ash	Fat	Protein	Carbohydrates (by difference)
Hegari.....	6.88	1.76	3.15	13.16	75.05
Dwarf yellow milo.....	6.64	1.78	3.37	13.87	74.34
Yellow corn.....	7.00	1.58	5.50	10.98	74.94

The figures in Table 1 do not differ materially from those of the United States Department of Agriculture (11). Drier air conditions in Arizona make an apparent higher protein, fat, and ash percentage of all the grains.

It is evident that chemical analysis gives no explanation of the observed superiority of yellow corn in the feeding of farm animals. The grain sorghums are distinctly higher than yellow corn in protein and lower in fat. The advantage of their higher protein content is, however, probably offset by a lower coefficient of digestibility (1). It may be that the higher fat content of the yellow corn is paralleled by a higher concentration of the fat-soluble vitamins and that this gives corn a distinct advantage over the sorghum grains.

ANIMAL-FEEDING EXPERIMENTATION

Unless otherwise indicated, all of the animals used in the following studies of the feeding value of the sorghum grains were of known nutritional history. They were taken from the stock colony of albino rats reared on Sherman's diet B, composed of two-thirds ground whole wheat, one-third whole milk powder, and sodium chloride equal to 2 per cent of the weight of the wheat, with fresh lettuce given daily. At the time of weaning (28 to 29 days) litter mates, matched as to size and sex as far as possible, were placed upon the different experimental rations. The living conditions of all the animals were identical, the animals being reared in square metal cages with false screen bottoms which were changed daily. Not more than three males and three females were kept in one cage. The experimental rations and distilled water were fed ad libitum. Weekly records of the weights of the animals and their food intake were kept, and a close observation of all the animals made, and all abnormalities recorded.

Pregnant females were separated from the lot and weighed every other day until their litters arrived, at which time their weights were again recorded. Bedding for the young consisted of finely cut white crêpe paper. The young were weighed as a lot weekly until they were 4 weeks old, at which time they were weighed separately, numbered and recorded as individuals, and the mother returned to the lot from which she came. For the purpose of studying the effect of the ration upon the succeeding generations, one pair of young from the first litter of each female was continued upon the diet of the mother.

Criteria for comparing the adequacy of the experimental rations consisted of such health factors as rapidity of growth, maximum size attained, age of maturity, success of reproduction and rearing of the young, physical vigor of parents and offspring, including duration of their prime of life (13, 14, 15, 16).

THE SORGHUM GRAINS AS THE SOLE SOURCE OF NUTRIMENT

The inadequacy of cereal grains as the sole source of nutriment for animals has long since been recognized. In 1915, McCollum and

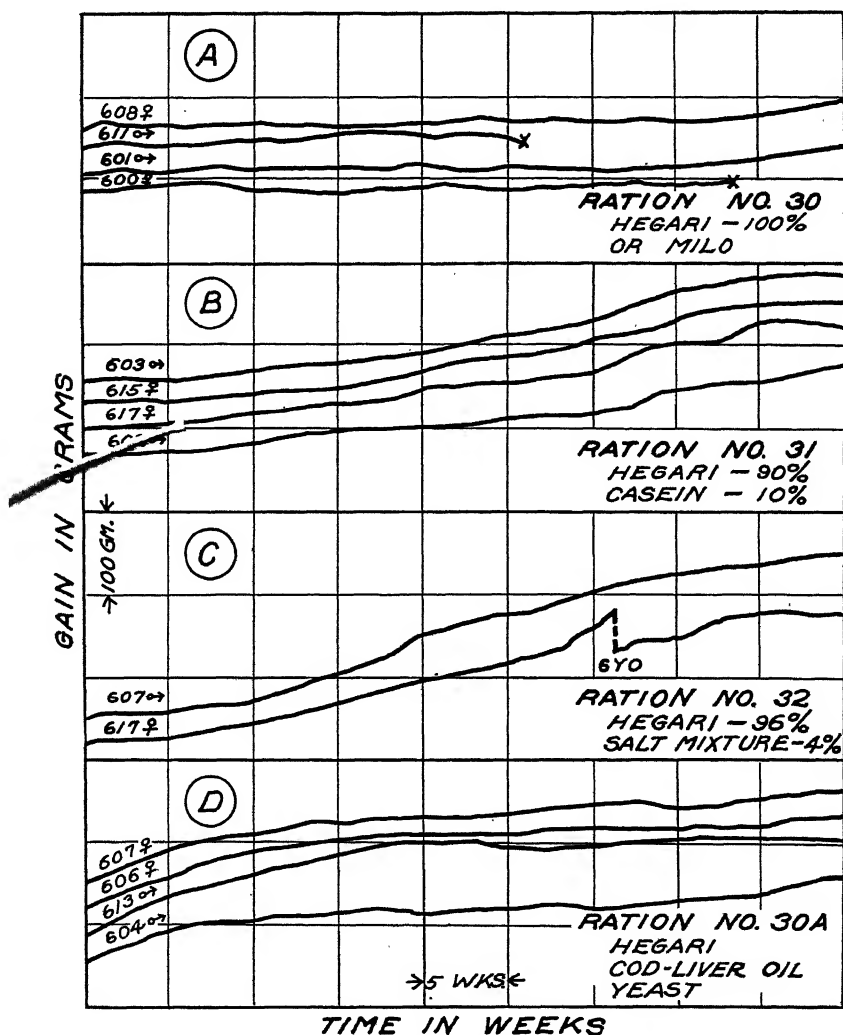


FIGURE 1.—Growth and reproduction records of representative animals receiving unsupplemented and supplemented hegari rations: A, Animals fed hegari or milo singly made no appreciable growth; B, growth response obtained by the addition of casein to the hegari ration; C, growth response obtained by the addition of Osborne and Mendel's salt mixture to the hegari ration; D, effect of supplementing hegari with cod-liver oil and yeast, in which case the slight growth response obtained may probably be due to the small amount of protein in the yeast

Davis (6) showed that wheat, oats, and corn when fed singly did not promote normal animal nutrition. Representative results of feeding hegari and yellow milo singly to albino rats from the time of weaning are shown graphically in Figure 1, A.

Both hegari and yellow milo are obviously deficient in dietary factors essential for growth or even continued maintenance through a normal life span. The animals in these groups made practically no gains in weight, nor showed much growth of the skeleton, and did not mature sexually. Two of the group died in the fifth and seventh months, respectively, and the others, though still living in the twelfth month, were in very poor physical condition. (Fig. 2.) On the other hand, when hegari is adequately supplemented, results such as appear in Figure 3 are obtained.

The growth performance of all the animals on ration 4 was superior to that of Donaldson's normal rat (2). The females matured early and produced four to five litters each during the 12 months of observation. The average size of the litters was 9.3. Ninety-two per cent of the young were successfully suckled and weaned at an average weight of 58 gm. No decline in health and vigor was seen in the

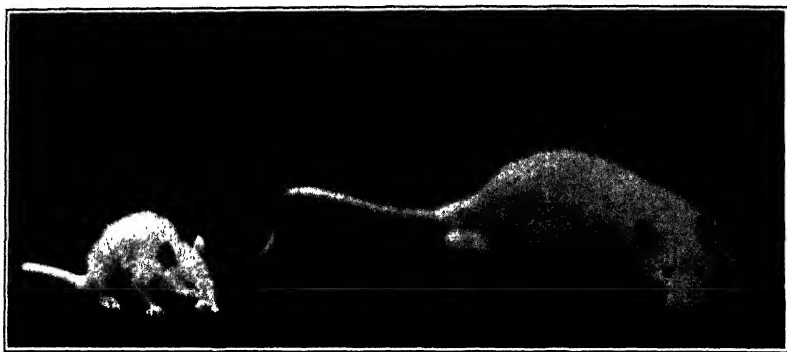


FIGURE 2.—Litter mates showing the effect of feeding hegari singly (left) and hegari suitably supplemented with protein, minerals, and vitamins (right)

fourth generation. Hegari when suitably supplemented apparently serves as a good basis for an adequate ration.

THE SORGHUM GRAINS AS SOURCES OF PROTEIN

McCollum and his associates (5, 7, 8, 9) has shown that certain grains, including wheat, oats, rice, and corn are inadequate in protein content, probably lacking certain essential amino acids in sufficient concentration. Heller and Green (3) have more recently indicated that milo does not provide sufficient protein for normal growth and, like corn, is incomplete in amino acid make-up. That protein is likewise a limiting factor in hegari is evident from the growth response obtained by the single addition of purified protein to the hegari ration (fig. 1, B), growth being resumed though at a subnormal rate when purified casein was added to the diet.

When hegari was fed as the chief source of protein in a diet which contained all other known dietary essentials, results such as appear in Figure 4 were obtained. Not only did the animals dependent upon 92 per cent hegari for their protein (ration 36)³ grow at a subnormal rate, but they reached maturity slowly. Some reproduction took place, but suckling of the young was markedly unsuccessful.

³ The small amount of protein in the yeast probably made the protein deficiency of hegari less apparent.

Of the 43 offspring born to two females during the period of observation, all but six died and were eaten by their mothers in the first and second week. The young that lived were capable of but very slow subsequent growth. They were undersized, weighing only 18 to 22 gm. at weaning, appeared well nourished though small, and

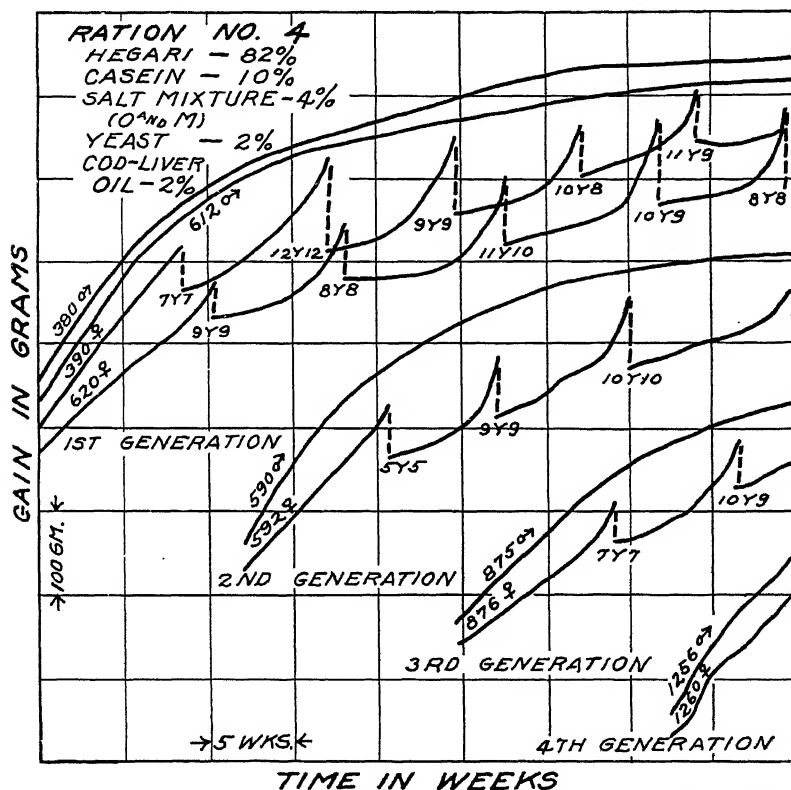


FIGURE 3.—Growth and reproduction records of representative animals receiving hegari adequately supplemented with protein, minerals, and vitamins. Growth at an optimum rate, health and vigor, and a high degree of success in reproduction and lactation resulted. Four generations were obtained in the year of observation. Each generation exhibited optimum growth and reproduced and reared young in their turn fully equal to the average. The break in the curves of females represents loss in weight upon parturition. The numeral before the Y indicates the number of offspring and that after the Y the number of young weaned

possessed the long lithe form that is characteristic of the stunting due to insufficient protein in the diet. (Fig. 5.)

THE SORGHUM GRAINS AS SOURCES OF MINERAL ELEMENTS

The meager growth acceleration resulting from the addition of protein to the ration of animals that had become stationary in weight when hegari was fed singly, suggested that protein is not the sole limiting factor of the sorghum grains. As early as 1889, Henry (4) noted the deficiency of the corn kernel in mineral elements. Since that time McCollum and his coworkers (5, 7, 8, 9) has demonstrated a deficiency of calcium, chlorine, and sodium in wheat, oats, rice, corn, and other seeds.

In Figure 1, C, may be seen the effect of feeding Osborne and Mendel's (10) salt mixture to animals that remained stationary in weight on the 100 per cent hegari ration. An immediate resumption of growth followed the addition of this mixture to the ration, though again the growth rate was below normal, due to other limiting factors. When hegari (86 per cent) served as the sole source of mineral elements in a diet which was otherwise complete (ration 33), data such

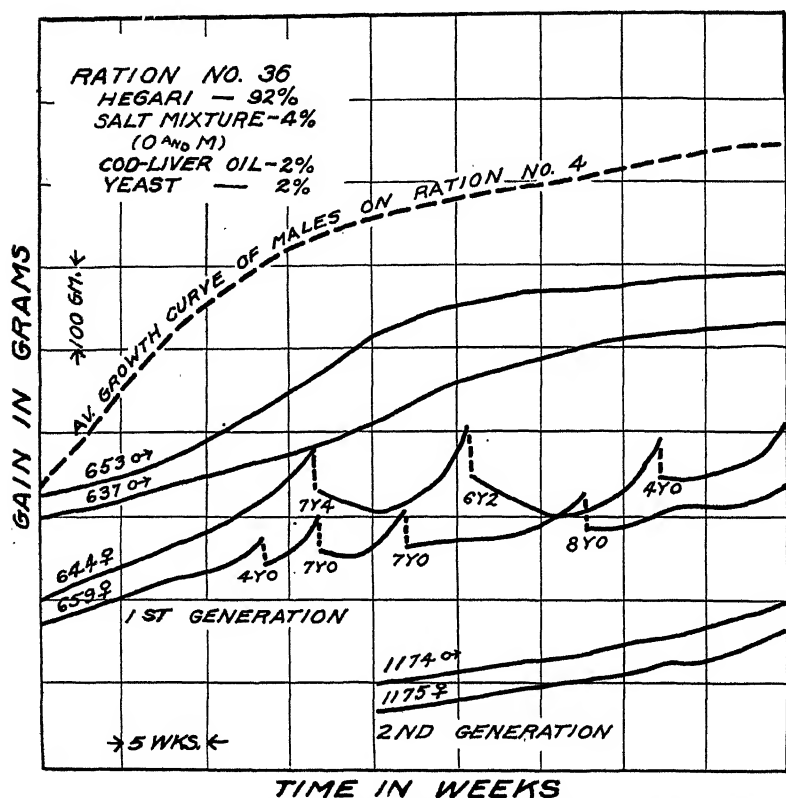


FIGURE 4.—Growth and reproduction records of representative animals receiving hegari as the chief source of protein in an otherwise complete ration; the growth rate is subnormal and reproduction highly unsuccessful

as appear in Figure 6 were obtained. All of the animals in this group made far less than normal weekly gains. Attainment of sexual maturity was delayed, and again reproduction and lactation were highly unsuccessful. Only 3 of 16 young born to 2 females were weaned and these were runts, weighing but 20 to 23 gm. Abnormal development of the skeleton was apparent, the animal presenting a short stocky appearance in contrast to the long lithe form of the normal or protein-stunted animals.

When sodium and chlorine were included in the ration in the form of 1 per cent sodium chloride (ration 38), growth was strikingly

accelerated and the reproduction record improved, though perfectly normal reproduction and lactation were not observed. (Fig. 7, A.) A more nearly optimum response, however, was obtained by the further addition of 1 per cent calcium lactate (ration 39), as shown in Figure 7, B. Undoubtedly hegari is deficient in calcium and in sodium or chlorine or both, and these must be introduced into rations of which hegari comprises a large part if normal growth and normal reproductive performance are to be expected.

THE SORGHUM GRAINS AS SOURCES OF VITAMIN A

On the basis of his studies of the nutritive value of wheat, barley, rice, oats, and corn, McCollum concluded that grains as a class are deficient in vitamin A and observed that vitamin A is concentrated in the more actively functioning part of the plant, that is, in the leaf or stem. On the other hand, Steenbock (18) has called attention to

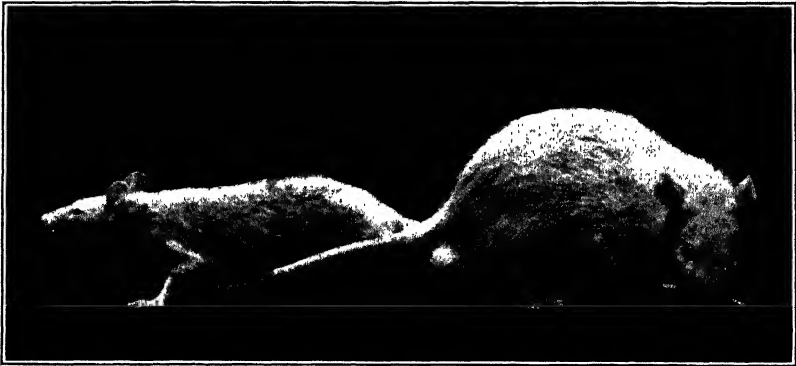


FIGURE 5.—Litter mates showing the protein inadequacy of hegari when constituting 92 per cent of the ration. Note the long, lithe form and poor coat of the animal (left) which was fed hegari as its sole source of protein, and compare with its normal litter mate (right) fed hegari and an adequate protein supplement

the association of vitamin A with yellow pigmentation and has shown that yellow corn, though a plant-storage organ, is rich in vitamin A, whereas white corn is strikingly deficient in this vitamin. In 1925, Heller and Green (3) reported that the feeding value of the sorghum grains is comparable to that of yellow corn, that the vitamin A content of most of the group is sufficient for all practical purposes, being present in amounts sufficient for growth and reproduction but in all species insufficient for continued rearing of the young.

In Figures 8 and 9 are graphically presented the growth and reproduction records of litter mates taken from the stock colony in this laboratory at the time of weaning and placed on rations in which vitamin A was derived from 84 per cent ground hegari (ration 37) or yellow corn (ration 3), respectively.

In Table 2, the average gains in weight of the males reared on rations 37 and 3 are compared with those of animals which received additional vitamin A in the form of cod-liver oil. (Ration 4, fig. 3.)

TABLE 2.—Average gains in weight of males on rations Nos. 37, 3, and 4

FIRST GENERATION

Ration No.	Number of males	Average initial weight	Average gain in weight in grams in—							
			4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks	28 weeks	36 weeks
		<i>Grams</i>								
37.....	9	57	102	151	195	227	244	272	276	276
3.....	4	58	113	185	236	264	270	289	316	319
4.....	6	57	112	189	254	277	294	312	327	342

SECOND GENERATION

37.....	7	37	73	139	202	233	245			
3.....	6	51	98	149	219	236	267	295	290	
4.....	6	56	109	178	250	272	290	306	320	

THIRD GENERATION

37.....	2	32	74							
3.....	4	44	98	162	203	238	251			
4.....	6	58	115	182	253	275	298			

FOURTH GENERATION

37 ^a										
3.....	2	42	96	166						
4.....	4	57	113	186						

^a No fourth generation on diet 37.

The rate of growth of the animals in both the unsupplemented hegari and corn groups is normal at first, the corn-fed animals, however, approaching the optimum growth rate more closely. The lot on hegari (ration 37) exhibits a greater slackening of the growth rate as adult size is approached, and none of the animals of the lot receiving additional vitamin A (ration 4) attain the maximum size for their age. It may be noted that growth in the successive generations on rations 37 and 3 was progressively more retarded, whereas the young of animals given a diet in which there was no lack of vitamin A showed no such slackening of the growth rate, even in the fourth generation.

As pointed out by Sherman and his associates (13, 14, 15, 16), differences in size are not of such great significance as the "differences in vigor reflected by breeding records." Reproductive records for one-third of the normal life span of the first generation females on rations 37, 3, and 4 are given in Table 3.

TABLE 3.—Reproductive records of first generation females on rations Nos. 37, 3, and 4

Ration No.	Number of litters per female	Average size of litters	Average number of young per female	Average number of young weaned	Percentage of young weaned	Average weight of young at weaning
						<i>Grams</i>
37.....	4	7.0	28.0	15.0	53.6	37
3.....	5	9.6	48.0	35.0	73.0	51
4.....	5	9.6	43.2	39.5	91.8	56

Though there is practically no difference in the number of litters produced by each female, a difference in size of the litters and a more striking difference in the percentage of young successfully suckled and weaned may be noted. The females reared on hegari ration 37 gave birth to litters with an average size of 7 and weaned but 53.6 per cent of the young as compared with an average litter of 9.6 offspring with 91.8 per cent successfully suckled under the same rigorous living conditions by each female which had been given an

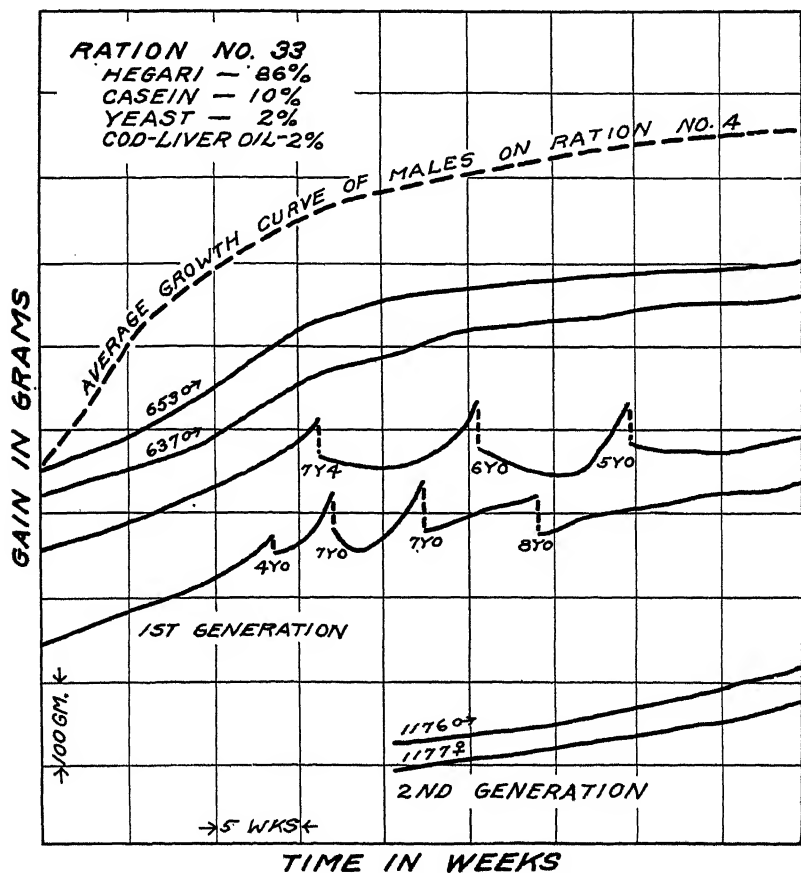


FIGURE 6.—Growth and reproduction records of representative animals receiving hegari as the chief source of mineral elements in an otherwise complete ration. All of the animals grew at a rate far below normal, reached sexual maturity slowly, and produced and raised but few young

additional source of vitamin A (ration No. 4). (Figs. 10 and 11.) It is also plain from Figure 8 that the percentage of young raised by each female on ration 37 falls sharply in the late litters, whereas no such corresponding drop in percentage reared is indicated in the records of females on ration 4, Figure 3. Continuing the animals on these rations for a longer period will therefore make this difference more striking, as a progressively smaller percentage of young weaned will be obtained in the group of animals dependent solely upon hegari for vitamin A. The difficulties of raising young and the

drain upon the mother rat were again indicated by the loss of her own body weight during the lactation period, as shown in Figure 7.

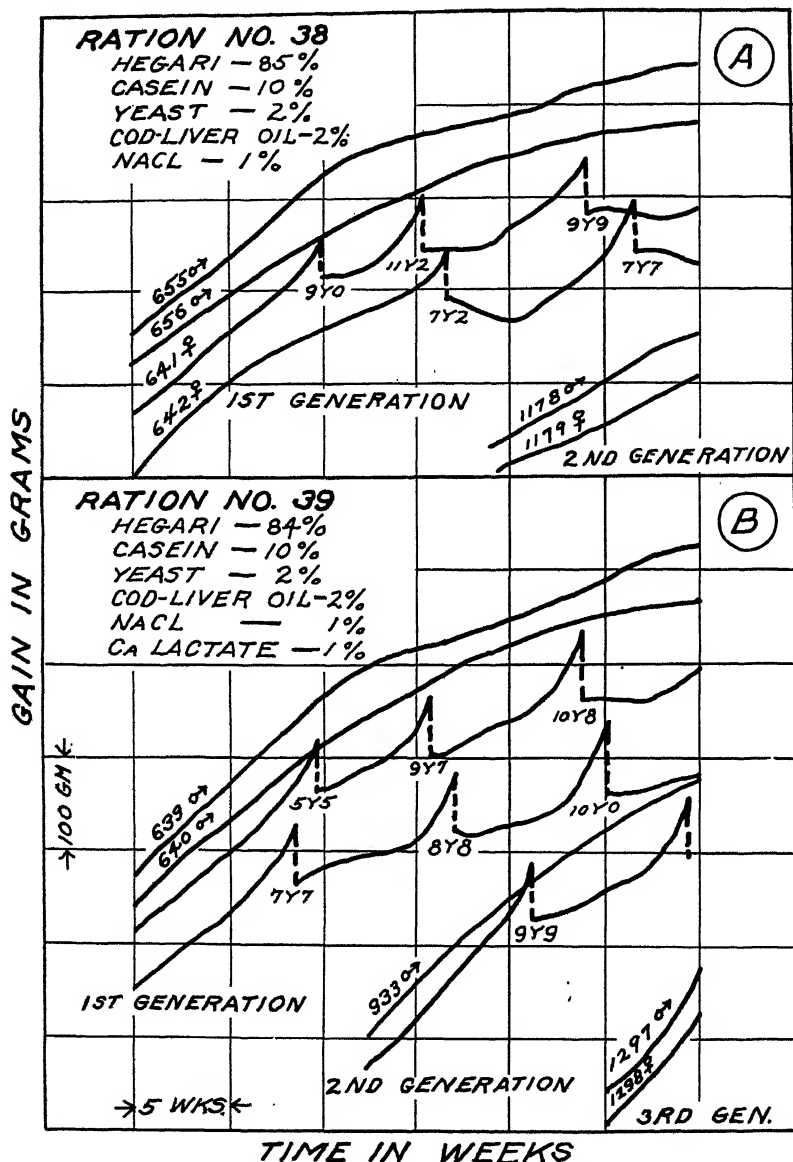


FIGURE 7.—A, Growth and reproduction records of representative animals that received sodium chloride in addition to hegari fed as the chief source of mineral elements in an otherwise complete ration; although marked improvement resulted from the inclusion of sodium chloride in the ration, perfectly normal reproduction was never obtained. B, The improved nutritional state of the animals on the further addition of calcium lactate to the ration indicates the deficiency of hegari in this respect.

By these same criteria, we have further proof of the superiority of yellow corn to hegari in vitamin-A content. The group of rats

dependent upon corn instead of hegari for vitamin not only produced larger litters but suckled a greater percentage of them and weaned them at a greater weight. However, it is evident that yellow corn does not provide enough vitamin A for continued rearing of offspring,

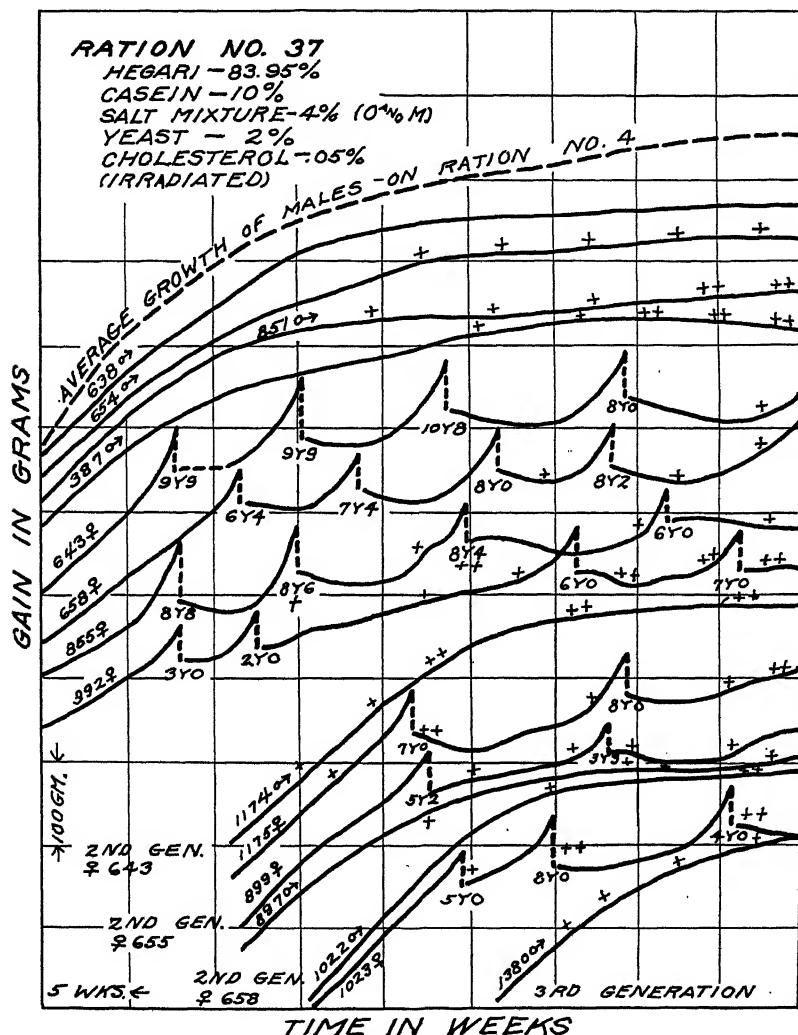


FIGURE 8.—Growth and reproduction records of representative animals receiving hegari as the chief source of vitamin A in an otherwise complete ration. None of the animals attained the rate of growth of the animals receiving additional vitamin A (note dotted curve). An early break in resistance to infection was indicated by the appearance of ophthalmia (note + signs). The females evidenced great difficulty in reproduction; they lost weight during the lactation period, and infant mortality was relatively high.

as shown by the inferior reproductive records in the later generations on ration 3.

Not only are hegari and yellow corn inadequate providers of sufficient vitamin A to induce optimum growth and reproduction but

apparently neither can be depended upon even to maintain animals in good health over a long period of time. A break in health and lowered resistance to infection were demonstrated by the appearance of positive signs of ophthalmia in both groups of animals. Here again, however, the corn-fed animals were the more fortunate, ophthalmia not appearing before the eleventh month in the first generation, whereas this characteristic symptom of vitamin-A deficiency appeared as early as the fifth month among the first-generation

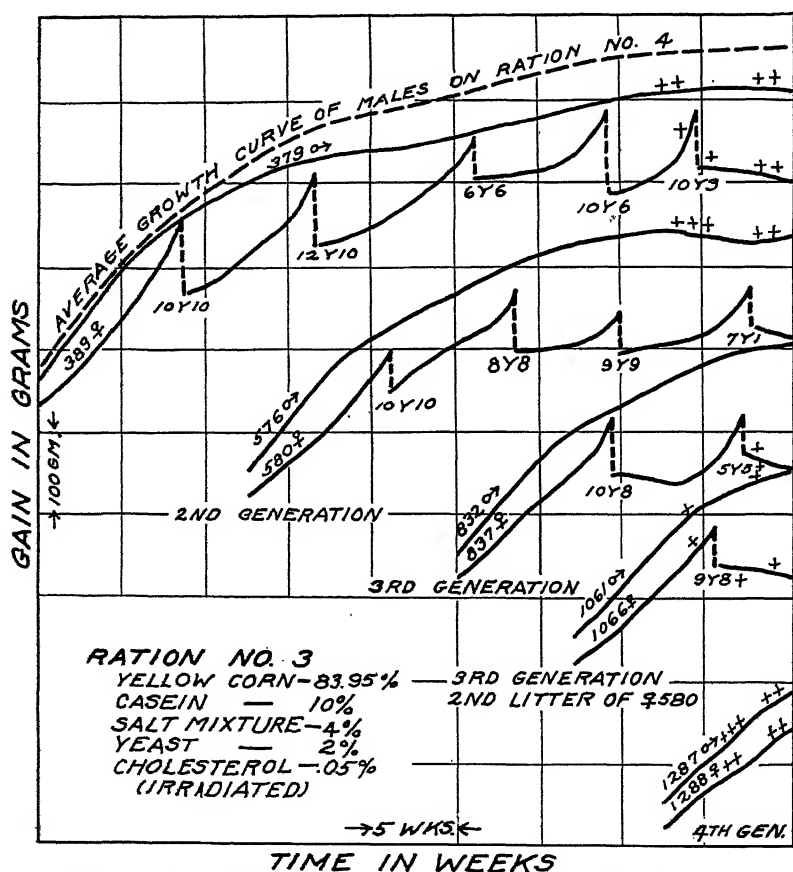


FIGURE 9.—Growth and reproduction records of representative animals receiving yellow corn as the chief source of vitamin A in an otherwise complete ration. Although these animals grew at an optimum rate at first they never attained the maximum adult size of the animals given additional vitamin A (note dotted curve). Ophthalmia (+ signs) appeared late in the first generation but earlier in each succeeding generation, and each successive generation was less capable of successful lactation.

animals dependent upon hegari for their vitamin A. Indication of respiratory infection evidenced by coughing, sneezing, and nasal discharge, and premature signs of old age were common in both groups, but a higher degree of health and vigor was obtained in the group reared upon the vitamin-A-supplemented hegari ration.

All of the evidences of the inadequate vitamin-A content of the hegari and corn rations are demonstrated more forcibly in the suc-

cessive generations. Not only is the rate of gain in the young lessened, the young not reaching the optimum size for their age, but the females are less productive and success in rearing their young is greatly reduced. The indications are that the families confined to hegari for vitamin A would soon die out. The second generation



FIGURE 10.—Female No. 876, reared on ration No. 4, with her litter of seven (fourth generation) normal, healthy young at 3 weeks of age

females were slower to attain sexual maturity and succeeded in raising comparatively few of their young (2 per cent). The young were stunted in size and physically inferior in every way. Positive signs of ophthalmia occurred early in the second generation and appeared in their young (third generation) before weaning in the

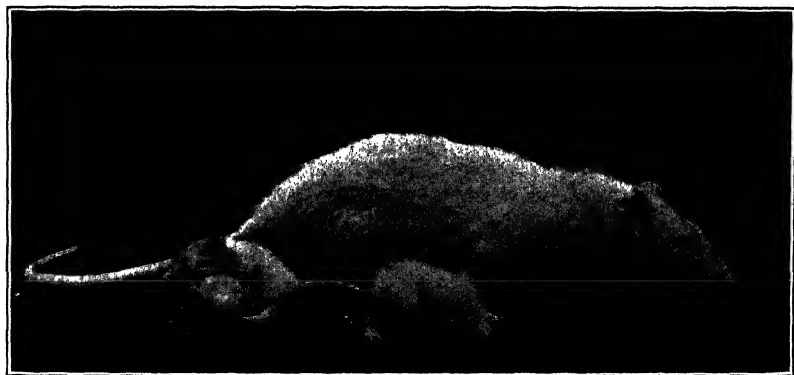


FIGURE 11.—Female No. 658, reared on ration No. 37, with her third litter. Although three of her original litter of eight (second generation) were alive at 3 weeks of age they were weak, stunted and under-developed

second week, showing a low vitamin-A content of the mother's milk. This eye condition in the young before weaning usually improved somewhat when the offspring began to partake of the mother's ration.

Not only was each generation inferior to the preceding one, but each successive litter was smaller in size, more susceptible to infection

and lower in reproductive capacity. Thus the first litter of female 855 averaged 37 gm. in weight and appeared physically fit, whereas the second litter averaged 30 gm. and showed ophthalmia at the time of weaning. In this respect also the superiority of yellow corn over hegari is unmistakable. However, though four generations of corn-fed animals were obtained during the 12 months' of experimental observation, each generation was less well equipped physically. The number of young born falls off as well as the percentage of offspring weaned, and eye infection appears earlier in each generation. Comparing corn and hegari in these respects, it is significant that the symptoms of lack of vitamin A in the fourth generation of corn-fed animals are of practically the same order as those appearing in the second generation of the hegari-fed group.

The inability of the young of each generation to develop as fully as those of the preceding generation may well be explained by the difference in the amount of vitamin A which was stored in their bodies. Vitamin A can be stored in large quantities, the amount depending upon the opportunity which the animal has for acquiring a reserve; that is, upon the concentration of vitamin A in the ration upon which the animal has fed and the length of time that it has had access to the ration (12, 17). It has been shown that the concentration of vitamin A in cow's milk is directly proportional to the concentration of the vitamin in the ration upon which the mother is maintained (1a). Animals at weaning may, therefore, possess a considerable store of vitamin A which would enable them to undergo a subsequent lack of this vitamin with less immediate ill effects and for a longer period of time than other animals of less fortunate dietary history.

There is ample reason for believing that the original animals used in this investigation possessed at weaning a reserve of vitamin A which fortified them for some time against a lack of this vitamin in the food upon which they were subsequently fed. That there was a gradual depletion of this initial surplus in the animals fed a ration in which either corn or hegari served as the sole source of vitamin A was demonstrated by the retardation of growth and failure in health as the animals matured on rations 37 and 3, and their later inability to raise their young. Their young were born with a smaller reserve of vitamin A and they had less opportunity to acquire any considerable store from their mother's milk. At weaning time, therefore, they were on a lower nutritional plane and less prepared to withstand any lack of vitamin A in their subsequent ration. The young of each successive litter were endowed with a smaller vitamin-A surplus and in turn had less to pass on to their young.

Obviously, then, such animal-feeding experiments as are here reported will fail of correct interpretation unless the storage factor be considered and the effect of a dietary régime studied over a long period of time. That differences in results of short-time feeding experiments under different conditions are to be expected is shown by the following experiments.

Young animals taken at the time of weaning from breeding rations of different vitamin-A content obtained by varying the percentage of alfalfa meal were placed on ration 37 and the response noted and compared. All of the animals used appeared physically fit and weighed from 50 to 65 gm. at weaning.

Group A consisted of a pair of 28-day-old rats whose mother had been reared on a ration containing 3 per cent of alfalfa meal. Group B and C mothers were raised on the same breeding ration, except that it contained 5 and 10 per cent of alfalfa meal, respectively. Group D, weaned from the stock colony, was given a diet entirely devoid of vitamin A⁴ for a period of four weeks, which time interval

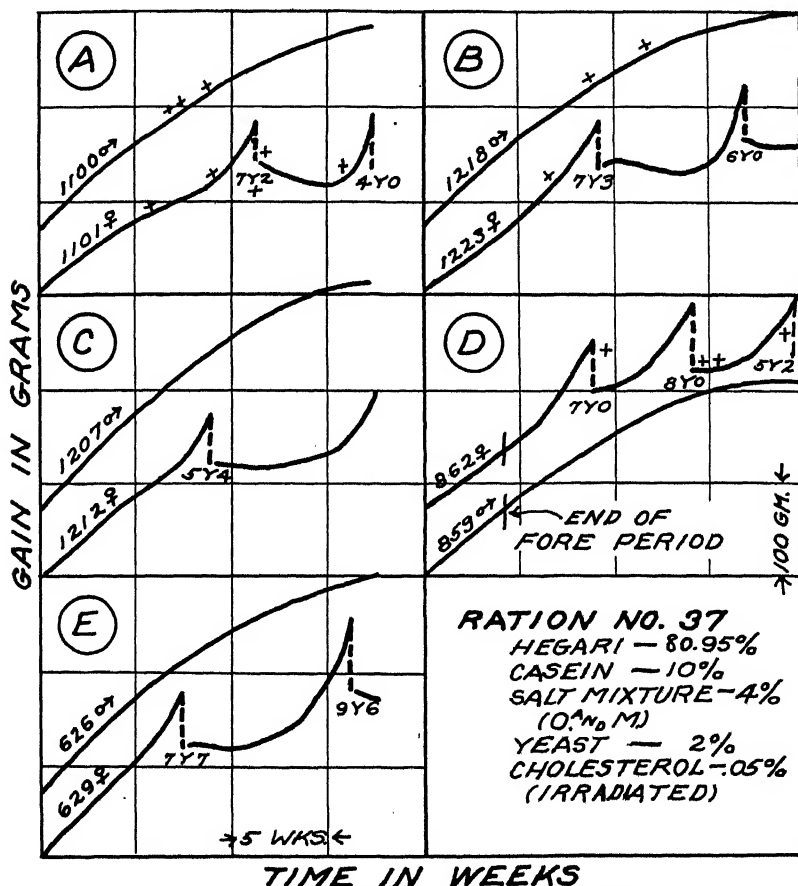


FIGURE 12.—Growth and reproduction records of animals possessing different stores of vitamin A, when placed upon a diet in which hegari served as the chief source of vitamin A. The opportunity for acquiring a reserve supply of vitamin A was least in pair A, only slightly greater in pair B, and twice as great in pair C. Pair D had their store of vitamin A exhausted before they were placed on ration 37. Pair E was taken from the stock colony at weaning and had, therefore, acquired a considerable store of vitamin A. Ophthalmia (+ sign) appears earlier and reproduction records are inferior for those animals possessing small vitamin-A reserves

has been proved many times practically to exhaust the existing vitamin-A stores in these animals. They were then confined to ration 37 in which hegari furnished all of the vitamin A. The results are shown in Figure 12.

⁴ The vitamin-A-free but otherwise adequate diet used in this laboratory consists of 18 per cent tetracted casein, 10 per cent dried yeast, 1 per cent NaCl, 4 per cent Osborne and Mendel's salt mixture, 66.95 per cent cornstarch, and 0.05 per cent irradiated cholesterol.

The unmistakable evidences of the inadequacy of vitamin A in ration 37 appeared earlier when the breeding rations of the animals contained less of this vitamin. Thus ophthalmia appeared in the sixth week in group A, in the eighth week in group B, and had not appeared in group C in the tenth week. Ophthalmia did not appear in the animals taken from the stock colony at weaning until the fifth month. It is again evident that hegari does not provide sufficient vitamin A for maintenance in health. If, however, the animals have an initial vitamin-A reserve, the inadequacy of the hegari ration will not be apparent until this surplus has been exhausted.

SUMMARY AND CONCLUSIONS

Comparative chemical analysis of hegari, yellow milo, and yellow corn shows the sorghums to be somewhat higher in protein and lower in fat than corn. This difference in chemical composition, however, does not satisfactorily explain the observed difference in nutritive value.

Like corn, hegari and milo when fed to rats as the sole source of nutriment do not promote normal development or even maintenance in health over a normal life span.

Like corn, hegari is inadequate in protein content. When fed to rats as the chief source of protein in an otherwise complete ration, growth is subnormal, sexual maturity delayed, and reproduction highly unsuccessful.

Like corn, hegari does not contain sufficient mineral elements to promote normal growth and reproduction and must be supplemented with sodium, chlorine, and calcium if normal nutrition is to be expected.

Yellow corn appears to be a far better source of vitamin A than hegari. Albino rats confined to a ration in which hegari served as the sole source of vitamin A in an otherwise adequate ration, grew at a fairly normal though not optimum rate and appeared to be in good health until their store of vitamin A was exhausted. The small amount of vitamin A in hegari, however, proved insufficient for continued growth at even a normal rate. All of the animals exhibited a low degree of health and vigor with pronounced susceptibility to infection and a marked failure in reproduction and rearing of their young. The indications are that families dependent on hegari for vitamin A would soon die out.

Hegari supplemented with suitable quantities of protein, mineral, and vitamin A not only promoted growth at an optimum rate and maintained the animals in every appearance of health and vigor, but also provided for the added nutritive demands of successful reproduction and lactation.

LITERATURE CITED

- (1) BALL, C. R., and ROTHEGEB, B. E.
1918. HOW TO USE SORGHUM GRAIN. U. S. Dept. Agr. Farmers' Bul. 972, 18 p., illus.
- (1a) CHICK, H., and ROSCOE, M. H.
1926. INFLUENCE OF DIET AND SUNLIGHT UPON THE AMOUNT OF VITAMIN A AND VITAMIN D IN THE MILK AFFORDED BY A COW. *Biochem. Jour.* 20: 632-649, illus.

- (2) DONALDSON, H. H.
1924. THE RAT; DATA AND REFERENCE TABLES FOR THE ALBINO RAT (*MUS NORVEGICUS ALBINUS*) AND THE NORWAY RAT (*MUS NORVEGICUS*). Ed. 2, 469 p., illus. Philadelphia. (Wistar Inst. Anat. and Biol. Mem. 6.)
- (3) HELLER, V. G., and GREEN, R.
1926. THE CHEMICAL AND NUTRITIVE PROPERTIES OF THE GRAIN SORGHUMS. *Jour. Metab. Research* 7-8: [205]-215, illus.
- (4) HENRY, W. A.
1889. EXPERIMENTS IN PIG FEEDING. BONE MEAL AND HARD WOOD ASHES, WITH CORN MEAL FOR HOGS. *Wis. Agr. Expt. Sta. Ann. Rpt.* 6: 15-17.
- (5) MCCOLLUM, E. V., and DAVIS, M.
1915. THE NATURE OF THE DIETARY DEFICIENCIES IN RICE. *Jour. Biol. Chem.* 23: 181-230, illus.
- (6) ——— and DAVIS, M.
1915. THE ESSENTIAL FACTORS IN THE DIET DURING GROWTH. *Jour. Biol. Chem.* 23: 231-246, illus.
- (7) ——— SIMMONDS, N., and PITZ, W.
1916. THE NATURE OF THE DIETARY DEFICIENCIES OF THE WHEAT EMBRYO. *Jour. Biol. Chem.* 25: 105-131, illus.
- (8) ——— SIMMONDS, N., and PITZ, W.
1916. DIETARY DEFICIENCIES OF THE MAIZE KERNEL. *Jour. Biol. Chem.* 28: 153-165, illus.
- (9) ——— SIMMONDS, N., and PITZ, W.
1917. THE NATURE OF THE DIETARY DEFICIENCIES OF THE OAT KERNEL. *Jour. Biol. Chem.* 29: 341-354, illus.
- (10) OSBORNE, T. B., and MENDEL, L. B., with the cooperation of FERRY, E. L. and WAKEMAN, A. J.
1919. THE NUTRITIVE VALUE OF THE WHEAT KERNEL AND ITS MILLING PRODUCTS. *Jour. Biol. Chem.* 37: 557-601, illus.
- (11) SCOTT, G. A.
1928. FEEDING GRAIN SORGHUMS TO LIVESTOCK. *U. S. Dept. Agr. Farmers' Bul.* 724, 10 p., illus.
- (12) SHERMAN, H. C., and CAMMACK, M. L.
1926. A QUANTITATIVE STUDY OF THE STORAGE OF VITAMIN A. *Jour. Biol. Chem.* 68: 69-74, illus.
- (13) ——— and CAMPBELL, H. L.
1924. GROWTH AND REPRODUCTION UPON SIMPLIFIED FOOD SUPPLY. IV. IMPROVEMENT IN NUTRITION RESULTING FROM AN INCREASED PROPORTION OF MILK IN THE DIET. *Jour. Biol. Chem.* 60: 5-15.
- (14) ——— and CROCKER, J.
1922. GROWTH AND REPRODUCTION UPON SIMPLIFIED FOOD SUPPLY. III. EFFICIENCY OF GROWTH AS INFLUENCED BY THE PROPORTION OF MILK IN THE DIET. *Jour. Biol. Chem.* 53: 49-52.
- (15) ——— and MUELFELD, M.
1922. GROWTH AND REPRODUCTION UPON SIMPLIFIED FOOD SUPPLY. II. INFLUENCE OF FOOD UPON MOTHER AND YOUNG DURING THE LACTATION PERIOD. *Jour. Biol. Chem.* 53: 41-47.
- (16) ——— ROUSE, M. E., ALLEN, B., and WOODS, E.
1921. GROWTH AND REPRODUCTION UPON SIMPLIFIED FOOD SUPPLY. I. *Jour. Biol. Chem.* 46: 503-519, illus.
- (17) ——— and STORMS, L. B.
1925. THE BODILY STORE OF VITAMIN A AS INFLUENCED BY AGE AND OTHER CONDITIONS. *Jour. Amer. Chem. Soc.* 47: 1653-1657, illus.
- (18) STEENBOCK, H.
1919. WHITE CORN VS. YELLOW CORN AND A PROBABLE RELATION BETWEEN THE FAT-SOLUBLE VITAMINE AND YELLOW PLANT PIGMENTS. *Science* (n. s.) 50: 352-353.
- (19) VINALL, H. N., GETTY, R. E., and CRON, A. B.
1924. SORGHUM EXPERIMENTS ON THE GREAT PLAINS. *U. S. Dept. Agr. Bul.* 1260, 88 p., illus.

A QUANTITATIVE COMPARISON OF THE VITAMIN-A CONTENT OF YELLOW CORN AND THE GRAIN SORGHUMS HEGARI AND YELLOW MILO¹

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INTRODUCTION

Nutritive tests of the comparative dietary value of the sorghum grains and yellow corn carried on in the author's laboratory at the University of Arizona and presented in a previous paper² have demonstrated qualitatively the superiority in vitamin-A content of yellow corn to the grain sorghums hegari and yellow milo. Both hegari and yellow milo when fed to rats as the sole source of vitamin A in an otherwise complete ration were shown to be far less able than yellow corn to promote normal growth, maintenance of health and vigor, and more especially, reproduction and successful rearing of the young.

It is the purpose of this paper to compare quantitatively the vitamin-A content of yellow corn and the grain sorghums hegari and yellow-milo.³

METHODS AND MATERIAL

Selected samples of hegari, dwarf yellow milo, and yellow corn grown on the university experimental farms and yellow corn grown in Riley County, Kans., were used in the tests.

The experimental method employed for the quantitative measurement of the vitamin-A content of the three grains was that developed by Sherman and Munsell.⁵ Young albino rats of known nutritional history⁶ taken at the time of weaning (28 days) from the stock colony were placed on a vitamin-A-free but otherwise adequate ration composed of 18 per cent casein, 10 per cent dried brewer's yeast, 4 per cent Osborne and Mendel's salt mixture, 1 per cent sodium chloride, and 67 per cent cornstarch. The casein was freed from vitamin A by three successive extractions of one hour with fresh boiling 95 per cent alcohol under a reflux condenser and filtered when hot by suction.

Vitamin D was incorporated into this ration either by irradiating the cornstarch spread out in thin layers by means of an ultra-violet lamp for a period of 30 minutes at a distance of 12 inches, or by the addition of 0.05 per cent of commercial cholesterol irradiated in the same fashion.⁷

¹ Received for publication July 24, 1929; issued June, 1930.

² SMITH, M. C. THE COMPARATIVE NUTRITIVE VALUE OF YELLOW CORN AND THE GRAIN SORGHUMS HEGARI AND YELLOW MILO. *Jour. Agr. Research* 40: 1129-1145, illus. 1930.

³ In 1919, Steenbock⁴ called attention to the association of vitamin A with yellow pigmentation and demonstrated that yellow corn is richer in vitamin A than white corn. Hegari is without yellow pigmentation but yellow milo contains pigment, located chiefly however in the epicarp.

⁴ STEENBOCK, H. WHITE CORN VS. YELLOW CORN AND A PROBABLE RELATION BETWEEN THE FAT-SOLUBLE VITAMINE AND YELLOW PLANT PIGMENTS. *Science (n. s.)* 50: 352-353. 1919.

⁵ SHERMAN, H. C., and MUNSELL, H. E. THE QUANTITATIVE DETERMINATION OF VITAMIN A. *Jour. Amer. Chem. Soc.* 47: 1639-1646, illus. 1925.

⁶ The stock colony in this laboratory was raised on Sherman's diet B, composed of two-thirds whole wheat, one-third whole milk powder, and sodium chloride equal to 2 per cent of the weight of the wheat. Fresh lettuce was given daily.

⁷ SHERMAN, H. G., and BURTIS, B. FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A. *Jour. Biol. Chem.* 78: 671-680. 1928.

When the animals became practically stationary in weight on this vitamin-A-free ration (between the fourth and fifth week) and showed other indications of the exhaustion of reserve of vitamin A present in their bodies at weaning time, they were placed in individual all-metal cages with raised screen bottoms to prevent access to excreta. The basal vitamin-A-free ration and fresh distilled water were given *ad libitum*. Graded, weighed amounts of the ground grains whose vitamin-A content was to be measured, were fed daily for eight weeks to rats which had been carefully matched as to litter, sex, and weight. At least one, and usually two, representative animals (negative controls) were taken from each litter and kept on the unsupplemented basal ration.

All of the animals were weighed weekly and records kept of their weight and food consumption. General health observations were made daily and notations made of the time of appearance and severity of such manifestations of vitamin-A deficiency as the eye disease ophthalmia, respiratory infections evidenced by sneezing, coughing, and nasal discharge, cutaneous malnutrition, and diarrhea. At the termination of the experimental period the animals were chloroformed, autopsies performed, and records made of such post-mortem evidences of infection due to lack of vitamin A as pus in the salivary glands, middle ear, sinuses, lungs, and bladder.

Growth response to the additions of weighed amounts of the grain supplements and the time of appearance and severity of the above-mentioned symptoms of vitamin-A deficiency were used as criteria of the amount of vitamin A present in the grains under test. The results were expressed in terms of units of vitamin A,⁸ a unit of vitamin A as defined by Sherman being that amount which when fed daily to a standard test animal (as prepared above) will promote an average gain of 25 gm. in 8 weeks.

EXPERIMENTAL DATA

MEASUREMENT OF VITAMIN A IN HEGARI

The results of feeding hegari in amounts of 2, 3, 4, 5, and 6 gm. as the sole source of vitamin A are given in Table 1. With the higher levels of feeding, adjustment in the basal vitamin-A-free ration was made to insure adequate protein and mineral intake, as the grains formed such a large part of the diet.

Animals taken from the stock colony at the time of weaning and given a vitamin-A-free but otherwise adequate ration were found to exhaust their store of vitamin A between the fourth and fifth week (average 33 days). When continued upon the unsupplemented vitamin-A-free ration (negative controls) there was immediate loss of weight followed by the appearance of ophthalmia, infections of the respiratory tract, cutaneous malnutrition, and often diarrhea. Death followed usually before the end of the eight weeks' experimental period in this group, the animals living on an average 36.3 days after the depletion of their vitamin-A reserves. Upon autopsy, all of the animals showed signs of severe infection in the glands at the base of the tongue and in the sinuses, middle ear, and bladder.

⁸ The unit as used here has a somewhat different value than that defined by Sherman because of the fact that the average weight of the animals at the end of the fore period is greater than that of Sherman's animals.

TABLE 1.—*Summarized results of feeding various amounts of hegari daily to rats as their sole source of vitamin A*

Amount of hegari fed daily	Animals used	Average initial weight	Average weight at end of fore period	Average gain or loss in weight in 8 weeks	Probable error of mean gains or losses	Average survival in test period	Remarks
Grams	Number	Grams	Grams	Grams		Days	
0	31	53.9	128	-43.8	±2.2	36.3	Ante-mortem and post-mortem evidences of severe infection.
2	8	53.7	142	-19.2	±3.6	49.6	Do.
3	15	51.3	131	-7.0	±2.6	53.8	Fully 75 per cent showed both ante-mortem and post-mortem signs of infection.
4	18	52.6	133	+12.1	±2.0	55.3	50 per cent showed more than one sign of infection.
5	8	57.2	134	+22.0	±1.3	56.0	Slight nasal discharge only infection noted.
6	5	51.8	135	+39.0	±3.4	56.0	No ante-mortem or post-mortem evidences of infection.

* Survived the entire period.

Supplementing the vitamin-A-free ration with 2 gm. of hegari daily did not cause much improvement. Half of the animals in the group fed 2 gm. of hegari daily as the sole source of vitamin A died before the close of the eight weeks' experimental period and the others lost weight. All of the animals evidenced ophthalmia in some degree (from + + to + + + +) and showed post-mortem symptoms of severe infection in at least two places, usually in the glands at the base of the tongue, sinuses, and middle ear. Except for the fact that more of these animals lived throughout the eight weeks of the experimental period and lost less weight, the results were little better than those obtained from the negative control animals.

Although approximately 25 per cent of the animals receiving 3 gm. of hegari daily in addition to the basal vitamin-A-free ration died before the end of the eight weeks' experimental period, an average gain in weight of 7 gm. was observed in this group. Fully three-fourths of the animals, however, showed both ante-mortem and post-mortem signs of severe infection. Apparently, 3 gm. of hegari fed daily does not furnish sufficient vitamin A to insure even a limited rate of growth or freedom from the severe infections which result from a lack of vitamin A.

Four grams of hegari fed daily as the only source of vitamin A in an otherwise complete ration permitted all but two of the animals to live throughout the test period. Although an average gain in weight of 12.1 gm. was recorded for this group, fully three-fourths of the animals had ophthalmia in some degree, and more than half of them showed at least two post-mortem evidences of infection, that of the bladder being relatively more frequent.

All the animals receiving 5 gm. of hegari daily lived the eight weeks' period and made an average gain of 22 gm. A small loss in weight in the last week of the period was noted, however. No severe signs of infection were observed either before or after death, though in 33 per cent of the cases there was noted a more than usual amount of thick mucus in the nasal passages. It would appear that 5 gm. of hegari fed daily contains slightly less than the amount of vitamin A necessary to insure the unit rate of gain in the standard test animal and give complete protection against infection.

Feeding hegari as the only source of vitamin A at the level of 6 gm. daily sufficed to induce an average gain in weight of 39 gm. in the experimental eight weeks' period. None of the animals showed infection of any kind nor any indication of insufficiency of vitamin A other than subnormal growth. Obviously 6 gm. of hegari contains more than one unit of vitamin A, as defined by Sherman.

Table 1 summarizes the data obtained by feeding graded amounts of hegari as the sole source of vitamin A in an otherwise complete diet. These same data are depicted graphically in Figure 1.

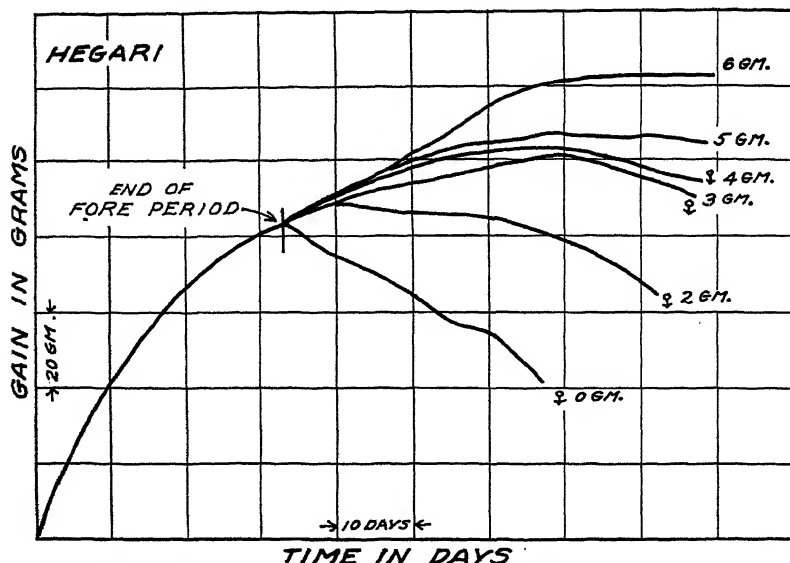


FIGURE 1.—Average gain curves of rats receiving various amounts of hegari daily as the sole source of vitamin A for a period of eight weeks

Obviously the unit⁸ rate of gain lies somewhere between that induced by feeding 5 and 6 gm. of hegari, respectively, as the only source of vitamin A in an otherwise adequate diet.

MEASUREMENT OF VITAMIN A IN YELLOW MILO

The results of feeding yellow milo as the sole source of vitamin A in amounts of 1, 1.5, 2, 2.5, 3, and 4 gm. appear in Table 2. The same data are presented graphically in Figure 2.

All of the animals fed only 1 gm. of yellow milo as a daily supplement to a vitamin-A-free, though otherwise adequate, diet lost weight (average 29.5 gm.), evidenced ophthalmia (+ + + +) before death, and showed extreme signs of infection upon autopsy. A slightly longer survival than the negative controls and less loss in weight are the only indications that the rats received any vitamin A.

The animals fed 1.5 gm. of milo daily survived considerably longer than those receiving no addition to their vitamin-A-free ration. However, 1.5 gm. of yellow milo did not prevent any of the animals in this group from dying as a result of lack of vitamin A. Ante-mortem and post-mortem symptoms of severe infection were present in every case.

⁸ The unit as used here has a somewhat different value than that defined by Sherman because of the fact that the average weight of the animals at the end of the fore period is greater than that of Sherman's animals.

TABLE 2.—*Summarized results of feeding various amounts of yellow milo daily to rats as their sole source of vitamin A*

Amount of milo fed daily	Animals used	Average initial weight	Average weight at end of fore-period	Average gain or loss in weight in 8 weeks	Probable error of mean gains or losses	Average survival in test	Remarks
Grams	Number	Grams	Grams	Grams		Days	
0	31	53.9	128.0	-43.8	± 2.2	36.3	Ante-mortem and post-mortem evidences of severe infection in all animals.
1.0	4	61.5	151.2	-29.5	± 5.7	48.0	Survival prolonged but animals were all severely infected.
1.5	7	59.8	133.0	-11.4	± 2.8	50.5	Do.
2.0	15	54.2	131.8	+11.4	± 2.6	52.8	25 per cent died during test period. 66 per cent of all animals had ophthalmia and pus sacks in tongue glands.
2.5	6	50.1	113.5	+21.6	± 2.6	* 56.0	Only one animal in this group had ophthalmia (+); no other sign of infection.
3.0	6	45.1	131.1	+23.5	± 1.1	* 56.0	No ante-mortem or post-mortem evidences of infections.
4.0	7	52.1	138.0	+43.7	± 2.0	* 56.0	Do.

* Survived the entire period.

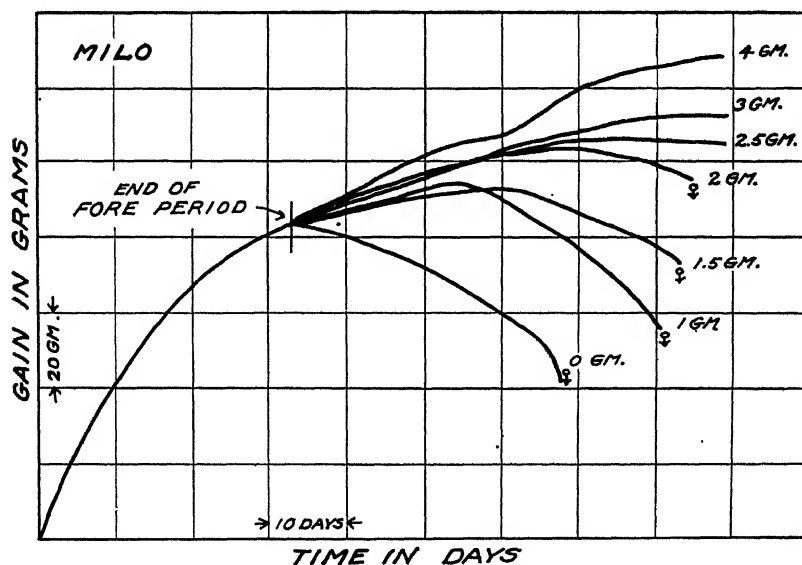


FIGURE 2.—Average gain curves of rats receiving various amounts of yellow milo daily as the sole source of vitamin A for a period of eight weeks

Two-thirds of the animals in the group fed 2 gm. of yellow milo daily in addition to their vitamin-A-free ration lived the eight weeks' of the experimental period, though they grew slowly. An average gain of 11.4 gm. in the test period was obtained. Sixty-six per cent of them, however, had infection resulting from insufficient vitamin A in their food.

Two and one-half grams of yellow milo fed daily furnishes sufficient vitamin A to induce an average rate of gain in the test animals of 21.6 gm. Most of the rats in this group were free from infection in those parts of the body which are commonly unable to resist the

invasion of bacteria in the absence of vitamin A in the ration. One rat, however, showed definite signs of ophthalmia in the last of the experimental period.

Three grams of yellow milo fed daily as the sole source of vitamin A not only induced an average rate of gain of 28.5 gm. in the eight weeks' experimental period, but also insured complete protection against infection in all of the animals.

An average gain of 4.37 gm. was obtained when 4 gm. of milo was fed daily to the standard test animals. No symptoms of vitamin-A deficiency were evident in this group.

From the results of these experiments it is apparent that slightly more than 2.5 gm. of dwarf yellow milo contain 1 unit of vitamin A.

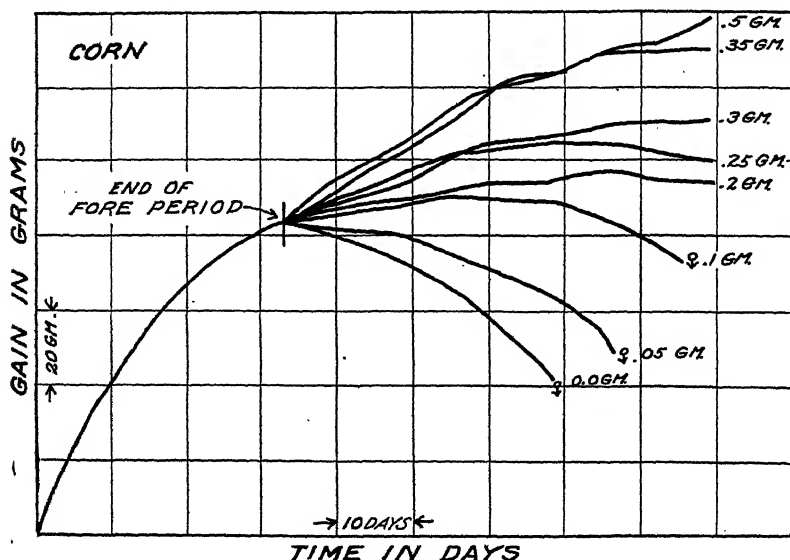


FIGURE 3.—Average gain curves of rats receiving various amounts of yellow corn daily as the sole source of vitamin A for period of eight weeks.

MEASUREMENT OF VITAMIN A IN YELLOW CORN

Results of feeding yellow corn in graded amounts of 0.05, 0.1, 0.2, 0.25, 0.3, 0.35, and 0.5 gm. as a daily supplement to a diet which contained no other source of vitamin A are presented in Table 3 and Figure 3.

Yellow corn fed daily in the amount of 0.05 gm. does not provide enough vitamin A to permit the standard test animals, which have no reserves of vitamin A, to live the experimental eight weeks' period. Every rat had ophthalmia (+ + to + + + +) by the fifth week and upon autopsy showed signs of infection of the same order as the negative control animals.

The animals fed 0.1 gm. of yellow corn daily lived longer than the negative controls (average 53.5 days), but they were not able to maintain themselves in health. Loss of weight occurred after the third week of the test period and marked susceptibility to infection followed in every case.

TABLE 3.—*Summarized results of feeding various amounts of yellow corn daily to rats as their sole source of vitamin A*

Amount of corn fed daily	Animals used	Average initial weight	Average weight at end of fore period	Average gain or loss in weight in 8 weeks	Probable error of mean gains or losses	Average survival in test period	Remarks
Grams 0	Number 31	Grams 53.9	Grams 128.0	Grams -43.8	± 2.2	Days 36.3	Ante-mortem and post-mortem evidences of severe infection in all animals.
.05	8	49.8	131.5	-35.2	± 2.6	43.0	Do.
.10	6	50.0	140.1	-10.3	± 4.1	53.5	75 per cent of the animals showed both ante-mortem and post-mortem signs of infection.
.20	4	58.2	137.0	+11.2	± 0.8	* 56.0	Definite though mild ophthalmia in 33 per cent of the animals.
.25	9	51.0	139.2	+17.1	± 2.2	* 56.0	No ante-mortem or post-mortem evidences of infection.
.30	5	54.2	125.2	+27.6	± 1.4	* 56.0	Do.
.35	2	58.0	136.0	+46.0	± 2.0	* 56.0	Do.
.50	2	42.0	120.0	+53.5	± 3.0	* 56.0	Do.

* Survived the entire period.

When 0.2 gm. of yellow corn was fed daily to rats as the only source of vitamin A in an otherwise adequate ration, a limited rate of gain (11.2 gm. in eight weeks) was made. It was not sufficient, however, to prevent a loss in weight after the sixth week or to insure the animal against infection, for more than 50 per cent of the animals in this group had definite though mild ophthalmia and pus in the glands at the base of the tongue.

The feeding of yellow corn at the level of 0.25 gm. daily induced an average rate of gain of 17.1 gm. in the eight weeks' test period. However, the initial rate of gain was not maintained throughout the whole experimental period and 33 per cent of the animals had definite though not severe (+) ophthalmia in the last week. Considerable variation in growth response was obtained in this group, as is to be expected when slightly less than one unit of vitamin A is fed daily.

Yellow corn fed at the level of 0.3 gm. daily or greater induced a definite rate of gain. Resistance to infection was relatively high, for in no case was ophthalmia or any post-mortem symptom of infection due to the lack of vitamin A evident.

An inspection of Table 2 shows that practically a unit of vitamin A is contained in 0.3 gm. of high grade yellow corn. Feeding yellow corn in this amount daily as the sole source of vitamin A resulted in a unit rate of gain in the test animals and freedom from infection.

From the results here reported it is apparent that yellow corn is a far better source of vitamin A than either hegari or yellow milo. It is evident also that yellow milo, contains more of this vitamin than hegari. The unit rate of gain (25 gm. in eight weeks) was induced by feeding slightly less than 0.3 gm. of yellow corn, 3 gm. of yellow milo, and somewhat more than 5 gm. of hegari. Practically the same rate of gain (11 gm.) in the test period was also obtained by feeding 0.2 gm. of yellow corn, 2 gm. of milo, and 4 gm. of hegari.

CONCLUSIONS

From the data obtained upon the quantitative measurement of the vitamin-A content of yellow corn and the sorghum grains hegari and yellow milo it may be concluded that the yellow milo is twice as rich in vitamin A as hegari, and that yellow corn is practically 20 times as rich a source of vitamin A as hegari.



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